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Remarks:
•This application was filed on 14-05-2019 as a divisional application to the application mentioned under INID code 62.
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(54) ALPHA (1,2) FUCOSYLTRANSFERASES SUITABLE FOR USE IN THE PRODUCTION OF FUCOSYLATED OLIGOSACCHARIDES

(57) The invention provides compositions and methods for engineering *E. coli* or other host production bacterial strains to produce fucosylated oligosaccharides, and the use thereof in the prevention or treatment of infection.

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Description

RELATED APPLICATIONS

[0001] This application claims priority to, and the benefit of, U.S. Patent Application No. 13/557,655, filed July 25, 2012, the contents of which are incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The invention provides compositions and methods for producing purified oligosaccharides, in particular certain fucosylated oligosaccharides that are typically found in human milk.

BACKGROUND OF THE INVENTION

[0003] Human milk contains a diverse and abundant set of neutral and acidic oligosaccharides. More than 130 different complex oligosaccharides have been identified in human milk, and their structural diversity and abundance is unique to humans. Although these molecules may not be utilized directly by infants for nutrition, they nevertheless serve critical roles in the establishment of a healthy gut microbiome, in the prevention of disease, and in immune function. Prior to the invention described herein, the ability to produce human milk oligosaccharides (HMOS) inexpensively was problematic. For example, their production through chemical synthesis was limited by stereo-specificity issues, precursor availability, product impurities, and high overall cost. As such, there is a pressing need for new strategies to inexpensively manufacture large quantities of HMOS.

SUMMARY OF THE INVENTION

[0004] The invention features an efficient and economical method for producing fucosylated oligosaccharides. Such production of a fucosylated oligosaccharide is accomplished using an isolated nucleic acid comprising a sequence encoding a lactose-utilizing α (1,2) fucosyltransferase gene product (e.g., polypeptide or protein), which is operably linked to one or more heterologous control sequences that direct the production of the recombinant fucosyltransferase gene product in a bacterium such as *Escherichia coli* (*E. coli*). In one example, the bacterium is an enteric bacterium. The amino acid sequence of the lactose-accepting α (1,2) fucosyltransferase gene product is preferably at least 10% and less than 40% identical to FutC (SEQ ID NO:2).

[0005] Also within the invention is a nucleic acid construct comprising an isolated nucleic acid encoding a lactose-accepting α (1,2) fucosyltransferase enzyme, said nucleic acid being operably linked to one or more heterologous control sequences that direct the production of the enzyme in a host bacteria production strain, wherein the amino acid sequence of the gene product (enzyme) encoded by the nucleic acid comprises about 70% identity to SEQ ID NO:2. For example, the construct comprises SEQ ID NO: 7, which encodes a FutL protein. By "heterologous" is meant that the control sequence and protein-encoding sequence originate from different bacterial strains. A suitable production host bacterial strain is one that is not the same bacterial strain as the source bacterial strain from which the fucosyltransferase-encoding nucleic acid sequence was identified.

[0006] A method for producing a fucosylated oligosaccharide, e.g., an HMOS, in a bacterium is carried out by providing a bacterium such as a production host strain, *Escherichia coli* (*E. coli*), that is characterized by a reduced level of β-galactosidase activity, a defective colonic acid synthesis pathway, a mutation in an ATP-dependent intracellular protease, a mutation in a *lacA* gene and an exogenous α (1,2) fucosyltransferase gene. Preferably, a mutation in a *thyA* gene in the host bacterium allows for the maintenance of plasmids that carry *thyA* as a selectable marker gene. Exemplary alternative selectable markers include antibiotic resistance genes such as BLA (beta-lactamase), or *proBA* genes (to complement a *proAB* host strain proline auxotropy) or *purC* (to complement a *purC* host strain adenine auxotrophy). The bacterium comprising these characteristics is cultured in the presence of lactose, and a fucosylated oligosaccharide is retrieved from the bacterium or from a culture supernatant of the bacterium. In some cases, the method further comprises culturing the bacterium in the presence of tryptophan and in the absence of thymidine. In preferred embodiments, the production host strain comprises *E. coli* K12. Other production host organisms are listed below.

[0007] The invention provides a purified fucosylated oligosaccharide produced by the methods described herein. The fucosylated oligosaccharide is purified for use in therapeutic or nutritional products, or the bacterium is used directly in such products. The fucosylated oligosaccharide produced by the engineered bacterium is 2'-fucosyllactose (2'-FL) or lactodifucotetraose (LDFT). The new alpha 1,2-fucosyltransferases are also useful to synthesize HMOS of larger molecular weight bearing alpha 1,2 fucose moieties, e.g., lacto-N-fucopentaose (LNF I) and lacto-N-difucohexaose (LDFH I).

[0008] The bacterium used to produce the oligosaccharides is genetically engineered to comprise an increased intracellular guanosine diphosphate (GDP)-fucose pool (compared to wild type), an increased intracellular lactose pool

(compared to wild type), and to comprise fucosyltransferase activity. Accordingly, an endogenous *lacZ* gene and an endogenous *lacI* gene of the *E. coli* are deleted or functionally inactivated to reduce the level of β-galactosidase activity. The bacterium may also comprise a mutation in the *lacA* gene. The isolated *E. coli* bacterium also comprises a *lacIq* gene promoter immediately upstream of a *lacY* gene. In some cases, the isolated *E. coli* bacterium comprises a defective colonic acid synthesis pathway due to an endogenous *wcaJ* gene of the *E. coli* being deleted or functionally inactivated. The bacterium comprises a mutation in an adenosine-5'-triphosphate (ATP)-dependant intracellular protease. For example, the bacterium comprises a null mutation in a *lon* gene. The bacterium also comprises a mutation in a *thyA* gene. Preferably, the bacterium accumulates an increased intracellular lactose pool and an increased intracellular GDP-fucose pool. In one aspect, the *E. coli* bacterium comprises the genotype Δ*ampC*::Ptrp^{B}c*I*, Δ(*lacI-lacZ*)::*FRT*, P_{lacIq}*lacY*$^{+}$, Δw-ca*J*::FRT, *thyA*::Tn*10,* Δ*lon*:(npt3, *lacZ*$^{+}$), *ΔlacA*.

[0009] The bacterium possesses fucosyl transferase activity. For example, the bacterium comprises an exogenous α (1,2) fucosyltransferase gene. Preferably, the exogenous α (1,2) fucosyltransferase gene comprises at least 10% homology/identity and less than 40% at the amino acid level to *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (*futC*), e.g., at least 15%, at least 20%, at least 25%, at least 30% identity. In other examples, the sequences are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% homology/identity to *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (*futC*). In one example, FutL is 70% identical to FutC at the amino acid level.

[0010] The term "% identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. For example, % identity is relative to the entire length of the coding regions of the sequences being compared.

[0011] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0012] Percent identity is determined using search algorithms such as BLAST and PSI-BLAST (Altschul et al., 1990, J Mol Biol 215:3, 403-410; Altschul et al., 1997, Nucleic Acids Res 25:17, 3389-402). For the PSI-BLAST search, the following exemplary parameters are employed: (1) Expect threshold was 10; (2) Gap cost was Existence: 11 and Extension:1; (3) The Matrix employed was BLOSUM62; (4) The filter for low complexity regions was "on". The bacterium expresses a fucosyltransferase gene product encoded by a sequence that is not identical to *futC.*

[0013] Exemplary α (1,2) fucosyltransferase genes include *Escherichia coli* 0126 *wbgL*, *Helicobacter mustelae* 12198 (ATCC 43772) alpha-1,2-fucosyltransferase (*futL*), and *Bacteroides vulgatus* ATCC 8482 glycosyl transferase family protein (*futN*). An exogenous α (1,2) fucosyltransferase gene is selected from the group consisting of *Escherichia coli* 0126 *wbgL*, *Helicobacter mustelae* 12198 (ATCC 43772) alpha-1,2-fucosyltransferase (*futL*), *Bacteroides vulgatus* ATCC 8482 glycosyl transferase family protein (*futN*), *Bacteroides fragilis* (NCTC) 9343 fucosyl transferase (*bft3lwcfB*), *Escherichia coli* O55:H7 (str. CB9615) fucosyltransferase (*wbgN*), *Helicobacter bilis* ATCC 437879 *futD*, *Vibrio cholera* 022 *wblA*, *Bacteroides fragilis* (NCTC) 9343 alpha-1,2-fucosyltransferase (*bft1*),

[0014] *Bacteroides ovatus* ATCC 8483 *futO*, and *Helicobacter cinaedi* CCUG 18818 alpha-1,2-fucosyltransferase (*futE*).

[0015] The invention also features a vector, e.g., a vector containing a nucleic acid. The vector can further include one or more regulatory elements, e.g., a heterologous promoter. The regulatory elements can be operably linked to a gene encoding a protein, a gene construct encoding a fusion protein gene, or a series of genes linked in an operon in order to express the fusion protein. In yet another aspect, the invention comprises an isolated recombinant cell, e.g., a bacterial cell containing an aforementioned nucleic acid molecule or vector. The nucleic acid is optionally integrated into the genome.

[0016] Also provided is a nucleic acid construct comprising at least one of a promoter of bacteriophage λ, an *E. coli rcsA* gene, a *bla* gene, and a native *thyA* gene. As an example of such a construct, the plasmid map of pG171 in Figure 5.

[0017] The sequence of pG171 is set forth below with annotations from GenBank regarding specific features (SEQ ID NO: 1):

LOCUS pEC2-futC-MYC-rcsA-thyA_(pG171) 6244 bp DNA
circular SYN 24-MAY-1995
DEFINITION Fusion cloning vector pTRXFUS, complete sequence.
ACCESSION U16857
VERSION U16857.1 GI:575447
KEYWORDS thioredoxin gene fusion vector.
SOURCE Cloning vector pTRXFUS (unknown)
ORGANISM Cloning vector pTRXFUS
other sequences; artificial sequences; vectors.

REFERENCE 1 (bases 1 to 3585)
AUTHORS LaVallie,E.R., DiBlasio,E.A., Kovacic,S., Grant,K.L.,
Schendel,P.F.
and McCoy,J.M.
TITLE A thioredoxin gene fusion expression system that circumvents inclusion body formation in the E. coli cytoplasm
JOURNAL Biotechnology (N.Y.) 11 (2), 187-193 (1993)
PUBMED 7763371
REFERENCE 2 (bases 1 to 3585)
AUTHORS LaVallie,E.R.
TITLE Direct Submission
JOURNAL Submitted (03-NOV-1994) Edward R. LaVallie, Genetics Institute,
87
CambridgePark Drive, Cambridge, MA 02140, USA

FEATURES Location/Qualifiers
Primer 163..183
/label=lacZR6

CDS 243..481
/gene="lgt" /note="ECK2824:JW2796:b2828"
/codon_start=1
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prolipoproteindiacylglyceryl
transferase"
/protein_id="BAE76897.1"
/db_xref="GI:85675644"
/translation="MTSSYLHFPEFDPVIFSIGPVALHWYGLMYLVGFIFAMWLATRRA

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MLFPGSRTEDILLLQTNPQWQSIFDTYGVLPRHPSQLYELLLEGVVLFIILNLYIRKPR	
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YRRSPQQHVS"	
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	/sub_strain="W3110"
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	/note="synonym: Escherichia coli str. K12 substr.
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Primer	243..266
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Promoter	359..364
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Promoter	380..385
	/label="thyA WEAK -10"
Binding_site	479..484
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Gene	488..1282
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CDS	488..1282
	/gene="thyA"

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   YQRSCDVFLGLPFNIASYALLVHMMAQQCDLEVGDFVWTGGDTHLYSNHMDQLSR
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     Primer                  complement (1468..1489)
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     Primer                  1508..1524
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     Gene                    1536..1588
                             /gene="dsrB"
     Primer                  1536..1558
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                             /label=rcsA-R
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QRPSVVFINEDCFIHDASNSQRIKLIINQHPNTLFIVFMAIANVHFDEYLLVRKNLLIS
SKSIKPESLDDILGDILKKETTITSFLNMPTLSLSRTESSMLRMWMAGQGTIQISDQMN
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Promoter complement (2393..2398)
/label=-10

Promoter complement (2419..2424)
/label=-35

Primer complement (2473..2495)
/note=cagtcagtcaaagcttCTACGAACATCTTCCAGGATACT
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Terminator complement (2502..2571)
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Primer complement (2553..2574)
/note=cagtcagtcaCTCGAGGCTGCAGTAATCGTACAGGGTAG
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Primer_binding_2575..2644
/PCR_primers=cagtcagtcactcgagtTTAattcaaatcttcttcagaaatcaatt
tttgttcAGCGTTATACTTTTGGGATTTTACCTC
/label="Primer 0011-futCMYC-4"

CDS complement (2574^2575)
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id"
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/gene=wcfW

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	/protein_id=CAH07600.1

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MHFRNYIISNSSFYWWGAWLSKYDDKLVIAPGNFINKDSVPESWFKLNVR

POLYLINKER	complement (2575..2580)
Protein	complement (2578..2581)
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Gene	complement (2582..3055)
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	/db_xref="GeneID:900162"
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Source	2582..3517
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	/mol_type="genomic DNA"
	/strain="26695"
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MYC-tag	complement (2585..2617)
Primer	2968..2990
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Primer	2968..2990
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Gene	complement (3052..3517)
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Primer	3541..3567
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	/label=pLnut-R
Region	complement (3544)
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note	3544
	/note="original sequenced pEC2-BfT2-MYC plasmid is a mixture of C and A here. This plasmid is A"
Misc._feature	complement (3547..3553)
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Misc._binding	complement (3600..3616)
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Region	complement (3601..3632)
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Variation	complement (3636..3715)
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     Misc._binding   complement (3987..4003)
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     Region          complement (3988..4018)
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     Source          complement (4024..4184)
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Region	complement (4024..4369)
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	/label="EC1 pL region"
mRNA	complement (4051)
	/note="mRNA-pl (alt.; via tl2a terminator)"
	/label="pL1 mRNA start"
Variation	4052..4184
	/note="imm434 region"
Promoter	complement (4058..4063)
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operator	complement (4060..4076)
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Primer	complement (4075..4092)
	/label=pLseq
Promoter	complement (4081..4086)
	/label="pL1 -35"
operator	complement (4084..4100)
	/note="operator-12 (first base on comp strand)"
	/label=OL2
mRNA	complement (4093)
	/label="pL2 mRNA start"
Promoter	complement (4100..4105)
	/label="pL2 -10"
operator	complement (4104..4120)
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Promoter	complement (4122..4127)
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Source	complement (4185..6244)

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Promoter	4853..4858
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RNA_transcript	4867..4974
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Promoter	complement (4988..4992)
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Promoter	complement (5008..5013)
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CDS	complement (5184..6044)
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Restriction_sit	5916..5927
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Signal-peptide	complement (5976..6044)
	/label=beta-lactamase
Promoter	complement (6088..6093)
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Promoter	complement (6109..6114)
	/label="beta-lactamase -35"

ORIGIN

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1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA
61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG
121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC
181 ACCATATATG CGGTGTGAAA TACCGCACAG ATGCGTAAGG AGAAAATACC GCATCAGGCG
241 CCTCCTCAAC CTGTATATTC GTAAACCACG CCCAATGGGA GCTGTCTCAG GTTTGTTCCT
301 GATTGGTTAC GGCGCGTTTC GCATCATTGT TGAGTTTTTC CGCCAGCCCG ACGCGCAGTT
361 TACCGGTGCC TGGGTGCAGT ACATCAGCAT GGGGCAAATT CTTTCCATCC CGATGATTGT
421 CGCGGGTGTG ATCATGATGG TCTGGGCATA TCGTCGCAGC CCACAGCAAC ACGTTTCCTG
481 AGGAACCATG AAACAGTATT TAGAACTGAT GCAAAAAGTG CTCGACGAAG GCACACAGAA
541 AAACGACCGT ACCGGAACCG GAACGCTTTC CATTTTTGGT CATCAGATGC GTTTTAACCT
601 GCAAGATGGA TTCCCGCTGG TGACAACTAA ACGTTGCCAC CTGCGTTCCA TCATCCATGA
661 ACTGCTGTGG TTTCTGCAGG GCGACACTAA CATTGCTTAT CTACACGAAA ACAATGTCAC
721 CATCTGGGAC GAATGGGCCG ATGAAAACGG CGACCTCGGG CCAGTGTATG GTAAACAGTG
781 GCGCGCCTGG CCAACGCCAG ATGGTCGTCA TATTGACCAG ATCACTACGG TACTGAACCA
841 GCTGAAAAAC GACCCGGATT CGCGCCGCAT TATTGTTTCA GCGTGGAACG TAGGCGAACT
901 GGATAAAATG GCGCTGGCAC CGTGCCATGC ATTCTTCCAG TTCTATGTGG CAGACGGCAA
961 ACTCTCTTGC CAGCTTTATC AGCGCTCCTG TGACGTCTTC CTCGGCCTGC CGTTCAACAT
1021 TGCCAGCTAC GCGTTATTGG TGCATATGAT GGCGCAGCAG TGCGATCTGG AAGTGGGTGA
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1081 TTTTGTCTGG ACCGGTGGCG ACACGCATCT GTACAGCAAC CATATGGATC AAACTCATCT
1141 GCAATTAAGC CGCGAACCGC GTCCGCTGCC GAAGTTGATT ATCAAACGTA AACCCGAATC
1201 CATCTTCGAC TACCGTTTCG AAGACTTTGA GATTGAAGGC TACGATCCGC ATCCGGGCAT
1261 TAAAGCGCCG GTGGCTATCT AATTACGAAA CATCCTGCCA GAGCCGACGC CAGTGTGCGT
1321 CGGTTTTTTT ACCCTCCGTT AAATTCTTCG AGACGCCTTC CCGAAGGCGC CATTCGCCAT
1381 TCAGGCTGCG CAACTGTTGG GAAGGGCGAT CGGTGCGGGC CTCTTCGCTA TTACGCCAGC
1441 TGGCGAAAGG GGGATGTGCT GCAAGGCGAT TAAGTTGGGT AACGCCAGGG TTTTCCCAGT
1501 CACGACGTTG TAAAACGACG GCCAGTGCCA AGCTTTCTTT AATGAAGCAG GGCATCAGGA
1561 CGGTATCTTT GTGGAGAAAG CAGAGTAATC TTATTCAGCC TGACTGGTGG GAAACCACCA
1621 GTCAGAATGT GTTAGCGCAT GTTGACAAAA ATACCATTAG TCACATTATC CGTCAGTCGG
1681 ACGACATGGT AGATAACCTG TTTATTATGC GTTTTGATCT TACGTTTAAT ATTACCTTTA
1741 TGCGATGAAA CGGTCTTGGC TTTGATATTC ATTTGGTCAG AGATTTGAAT GGTTCCCTGA
1801 CCTGCCATCC ACATTCGCAA CATACTCGAT TCGGTTCGGC TCAATGATAA CGTCGGCATA
1861 TTTAAAAACG AGGTTATCGT TGTCTCTTTT TTCAGAATAT CGCCAAGGAT ATCGTCGAGA
1921 GATTCCGGTT TAATCGATTT AGAACTGATC AATAAATTTT TTCTGACCAA TAGATATTCA
1981 TCAAAATGAA CATTGGCAAT TGCCATAAAA ACGATAAATA ACGTATTGGG ATGTTGATTA
2041 ATGATGAGCT TGATACGCTG ACTGTTAGAA GCATCGTGGA TGAAACAGTC CTCATTAATA
2101 AACACCACTG AAGGGCGCTG TGAATCACAA GCTATGGCAA GGTCATCAAC GGTTTCAATG
2161 TCGTTGATTT CTCTTTTTTT AACCCCTCTA CTCAACAGAT ACCCGGTTAA ACCTAGTCGG
2221 GTGTAACTAC ATAAATCCAT AATAATCGTT GACATGGCAT ACCCTCACTC AATGCGTAAC
2281 GATAATTCCC CTTACCTGAA TATTTCATCA TGACTAAACG GAACAACATG GGTCACCTAA
2341 TGCGCCACTC TCGCGATTTT TCAGGCGGAC TTACTATCCC GTAAAGTGTT GTATAATTTG
2401 CCTGGAATTG TCTTAAAGTA AAGTAAATGT TGCGATATGT GAGTGAGCTT AAAACAAATA
2461 TTTCGCTGCA GGAGTATCCT GGAAGATGTT CGTAGaagct tACTGCTCAC AAGAAAAAAG
2521 GCACGTCATC TGACGTGCCT TTTTTATTTG TACTACCCTG TACGATTACT GCAGCTCGAG
2581 TTTAattcaa atcttcttca gaaatcaatt tttgttcAGC GTTATACTTT TGGGATTTTA
2641 CCTCAAAATG GGATTCTATT TTCACCCACT CCTTACAAAG GATATTCTCA TGCCCAAAAA
2701 GCCAGTGTTT GGGGCCAATA ATGATTTTTT CTGGATTTTC TATCAAATAG GCCGCCCACC
2761 AGCTATAAGT GCTATTAGCG ATAATGCCAT GCTGACAAGA TTGCATGAGC AGCATGTCCC
2821 AATACGCCTC TTCTTCTTTA TCCCTAGTGG TCATGTCCAT AAAAGGGTAG CCAAGATCAA
2881 GATTTTGCGT GAATTCTAAG TCTTCGCAAA ACACAAAAAG CTCCATGTTT GGCACGCGCT
2941 TTGCCATATA CTCAAGCGCC TTTTTTTGAT AGTCAATACC AAGCTGACAG CCAATCCCCA
3001 CATAATCCCC TCTTCTTATA TGCACAAACA CGCTGTTTTT AGCGGCTAAA ATCAAAGAAA
3061 GCTTGCACTG ATATTCTTCC TCTTTTTTAT TATTATTCTT ATTATTTTCG GGtGGtGGtG
3121 GTAGAGTGAA GGTTTGCTTG ATTAAAGGGG ATATAGCATC AAAGTATCGT GGATCTTGGA
3181 AATAGCCAAA AAAATAAGTC AAGCGGCTTG GCTTTAGCAA TTTAGGCTCG TATTCAAAAA
3241 CGATTTCTTG ACTCACCCTA TCAAATCCCA TGCATTTGAG CGCGTCTCTT ACTAGCTTGG
3301 GGAGGTGTTG CATTTTAGCT ATAGCGATTT CTTTCGCGCT CGCATAGGGC AAATCAATAG
3361 GGAAAAGTTC TAATTGCATT TTCCTATCGC TCCAATCAAA AGAAGTGATA TCTAACAGCA
3421 CAGGCGTATT AGAGTGTTTT TGCAAACTTT TAGCGAAAGC GTATTGAAAC ATTTGATTCC
3481 CAAGCCCTCC GCAAATTTGC ACCACCTTAA AAGCCATATG tatatctcct tcttgaaTTC

3541 TAAaAATTGA TTGAATGTAT GCAAATAAAT GCATACACCA TAGGTGTGGT TTAATTTGAT
3601 GCCCTTTTTC AGGGCTGGAA TGTGTAAGAG CGGGGTTATT TATGCTGTTG TTTTTTTGTT
3661 ACTCGGGAAG GGCTTTACCT CTTCCGCATA AACGCTTCCA TCAGCGTTTA TAGTTAAAAA
3721 AATCTTTCGG AACTGGTTTT GCGCTTACCC CAACCAACAG GGGATTTGCT GCTTTCCATT
3781 GAGCCTGTTT CTCTGCGCGA CGTTCGCGGC GGCGTGTTTG TGCATCCATC TGGATTCTCC
3841 TGTCAGTTAG CTTTGGTGGT GTGTGGCAGT TGTAGTCCTG AACGAAAACC CCCCGCGATT
3901 GGCACATTGG CAGCTAATCC GGAATCGCAC TTACGGCCAA TGCTTCGTTT CGTATCACAC
3961 ACCCCAAAGC CTTCTGCTTT GAATGCTGCC CTTCTTCAGG GCTTAATTTT TAAGAGCGTC
4021 ACCTTCATGG TGGTCAGTGC GTCCTGCTGA TGTGCTCAGT ATCACCGCCA GTGGTATTTA
4081 TGTCAACACC GCCAGAGATA ATTTATCACC GCAGATGGTT ATCTGTATGT TTTTTATATG
4141 AATTTATTTT TTGCAGGGGG GCATTGTTTG GTAGGTGAGA GATCAATTCT GCATTAATGA
4201 ATCGGCCAAC GCGCGGGGAG AGGCGGTTTG CGTATTGGGC GCTCTTCCGC TTCCTCGCTC
4261 ACTGACTCGC TGCGCTCGGT CGTTCGGCTG CGGCGAGCGG TATCAGCTCA CTCAAAGGCG
4321 GTAATACGGT TATCCACAGA ATCAGGGGAT AACGCAGGAA AGAACATGTG AGCAAAAGGC
4381 CAGCAAAAGG CCAGGAACCG TAAAAAGGCC GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC
4441 CCCCCTGACG AGCATCACAA AAATCGACGC TCAAGTCAGA GGTGGCGAAA CCCGACAGGA
4501 CTATAAAGAT ACCAGGCGTT TCCCCCTGGA AGCTCCCTCG TGCGCTCTCC TGTTCCGACC
4561 CTGCCGCTTA CCGGATACCT GTCCGCCTTT CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT
4621 AGCTCACGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG
4681 CACGAACCCC CCGTTCAGCC CGACCGCTGC GCCTTATCCG GTAACTATCG TCTTGAGTCC
4741 AACCCGGTAA GACACGACTT ATCGCCACTG GCAGCAGCCA CTGGTAACAG GATTAGCAGA
4801 GCGAGGTATG TAGGCGGTGC TACAGAGTTC TTGAAGTGGT GGCCTAACTA CGGCTACACT
4861 AGAAGGACAG TATTTGGTAT CTGCGCTCTG CTGAAGCCAG TTACCTTCGG AAAAAGAGTT
4921 GGTAGCTCTT GATCCGGCAA ACAAACCACC GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG
4981 CAGCAGATTA CGCGCAGAAA AAAAGGATCT CAAGAAGATC CTTTGATCTT TTCTACGGGG
5041 TCTGACGCTC AGTGGAACGA AAACTCACGT TAAGGGATTT TGGTCATGAG ATTATCAAAA
5101 AGGATCTTCA CCTAGATCCT TTTAAATTAA AAATGAAGTT TTAAATCAAT CTAAAGTATA
5161 TATGAGTAAA CTTGGTCTGA CAGTTACCAA TGCTTAATCA GTGAGGCACC TATCTCAGCG
5221 ATCTGTCTAT TTCGTTCATC CATAGTTGCC TGACTCCCCG TCGTGTAGAT AACTACGATA
5281 CGGGAGGGCT TACCATCTGG CCCCAGTGCT GCAATGATAC CGCGAGACCC ACGCTCACCG
5341 GCTCCAGATT TATCAGCAAT AAACCAGCCA GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT
5401 GCAACTTTAT CCGCCTCCAT CCAGTCTATT AATTGTTGCC GGGAAGCTAG AGTAAGTAGT
5461 TCGCCAGTTA ATAGTTTGCG CAACGTTGTT GCCATTGCTA CAGGCATCGT GGTGTCACGC
5521 TCGTCGTTTG GTATGGCTTC ATTCAGCTCC GGTTCCCAAC GATCAAGGCG AGTTACATGA
5581 TCCCCCATGT TGTGCAAAAA AGCGGTTAGC TCCTTCGGTC CTCCGATCGT TGTCAGAAGT
5641 AAGTTGGCCG CAGTGTTATC ACTCATGGTT ATGGCAGCAC TGCATAATTC TCTTACTGTC
5701 ATGCCATCCG TAAGATGCTT TTCTGTGACT GGTGAGTACT CAACCAAGTC ATTCTGAGAA
5761 TAGTGTATGC GGCGACCGAG TTGCTCTTGC CCGGCGTCAA TACGGGATAA TACCGCGCCA
5821 CATAGCAGAA CTTTAAAAGT GCTCATCATT GGAAAACGTT CTTCGGGGCG AAAACTCTCA
5881 AGGATCTTAC CGCTGTTGAG ATCCAGTTCG ATGTAACCCA CTCGTGCACC CAACTGATCT
5941 TCAGCATCTT TTACTTTCAC CAGCGTTTCT GGGTGAGCAA AAACAGGAAG GCAAAATGCC

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6001 GCAAAAAAGG GAATAAGGGC GACACGGAAA TGTTGAATAC TCATACTCTT CCTTTTTCAA
6061 TATTATTGAA GCATTTATCA GGGTTATTGT CTCATGAGCG GATACATATT TGAATGTATT
6121 TAGAAAAATA AACAAATAGG GGTTCCGCGC ACATTTCCCC GAAAAGTGCC ACCTGACGTC
6181 TAAGAAACCA TTATTATCAT GACATTAACC TATAAAAATA GGCGTATCAC GAGGCCCTTT
6241 CGTC (SEQ ID NO:1; plasmid G171)
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[0018] The nucleic acid construct further comprises an α (1,2) fucosyltransferase gene comprising, e.g., at least 10% and less than 40% identity at the amino acid level to *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (*futC*). For example, the exogenous α (1,2) fucosyltransferase gene is selected from the group consisting of *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (*futC*), *Vibrio cholera 022 wblA*, *Escherichia coli* 0126 *wbgL*, *Helicobacter bilis* ATCC 437879 *futD*, *Helicobacter cinaedi* CCUG 18818 alpha-1,2-fucosyltransferase (*futE*), *Helicobacter mustelae* 12198 (ATCC 43772) alpha-1,2-fucosyltransferase (*futL*), *Bacteroides vulgatus* ATCC 8482 glycosyl transferase family protein (*futN*), *Bacteroides ovatus* ATCC 8483 *futO*, *Escherichia coli* O55:H7 (str. CB9615) fucosyltransferase (*wbgN*), *Bacteroides fragilis* (NCTC) 9343 alpha-1,2-fucosyltransferase (*bft1*), and *Bacteroides fragilis* (NCTC) 9343 fucosyl transferase (*bft3*/*wcfB*). The depiction of pG171 bears the alpha 1,2 FT *genefutC* to serve as an example.

[0019] Also within the invention is an isolated *E. coli* bacterium as described above and characterized as comprising a reduced level of β-galactosidase activity, a defective colonic acid synthesis pathway, a mutation in the *lacA* gene, a mutation in an ATP-dependant intracellular protease, and a mutation in a *thyA* gene. The invention also provides methods of identifying an α (1,2) fucosyltransferase gene capable of synthesizing 2'-fucosyllactose (2'-FL) in *E. coli.* The method of identifying non-FutC lactose-utilizing, α(1,2)fucosyltransferase enzyme comprises the following steps:

1) performing a computational search of sequence databases to define a broad group of simple sequence homologs of any known, lactose-utilizing α(1,2)fucosyltransferase;
2) using the list from step (1), deriving a search profile containing common sequence and/or structural motifs shared by the members of the list;
3) searching sequence databases, using a derived search profile based on the common sequence or structural motif from step (2) as query, and identifying a candidate sequences, wherein a sequence homology to a reference lactose-utilizing α(1,2)fucosyltransferase is 40% or less;
4) compiling a list of candidate organisms, said organisms being characterized as expressing α(1,2)fucosyl- glycans in a naturally-occurring state;
5) selecting candidate sequences that are derived from candidate organisms to generate a list of candidate lactose-utilizing enzymes;
6) expressing the candidate lactose- utilizing enzyme in a host organism; and
7) testing for lactose- utilizing α(1,2)fucosyltransferase activity, wherein detection of 2'-FL in said organism indicates that the candidate sequence comprises a non-FutC lactose-utilizing α(1,2)fucosyltransferase. For example, the sequence homology to a reference lactose- utilizing α(1,2)fucosyltransferase is 40% or less.

[0020] A purified fucosylated oligosaccharide produced by the methods described above is also within the invention. The purified oligosaccharide (2'-FL) obtained at the end of the process is a white/slightly off-white, crystalline, sweet powder. Unlike oligosaccharide production methods using FutC, the methods utilizing certain non-FutC enzymes (e.g. FutL) do not possess α (1,3) fucosyltransferase activity which leads to side reactions. The lack of α (1,3) fucosyltransferase activity associated with FutL contributes to its efficiency in producing 2'FL and is an advantage compared to FutC. FutL does not possess alpha 1,3 fucosyltransferase activity. For example, an engineered E.coli cell, E.coli culture supernatant, or E.coli cell lysate according to the invention comprises recombinant 2'-FL and does not substantially comprise a 1,3 fucosylated lactose prior to purification of 2'-FL from the cell, culture supernatant, or lysate. Both FutN and WbgL appear to have alpha 1,3 fucosyltransferase activity (similar to FutC). However as a general matter, the fucosylated oligosaccharide produced by the methods contains a negligible amount of 3-FL in a 2'-FL-containing cell, cell lysate or culture, or supernatant, e.g., less than 1% of the level of 2'-FL or 0.5% of the level of 2'-FL.

[0021] A purified oligosaccharide, e.g., 2'-FL or LDFT, is one that is at least 90%, 95%, 98%, 99%, or 100% (w/w) of the desired oligosaccharide by weight. Purity is assessed by any known method, e.g., thin layer chromatography or other chromatographic techniques known in the art. The invention includes a method of purifying a fucosylated oligosaccharide produced by the genetically engineered bacterium described above, which method comprises separating the desired fucosylated oligosaccharide (e.g., 2'-FL) from contaminants in a bacterial cell lysate or bacterial cell culture supernatant of the bacterium.

[0022] The oligosaccharides are purified and used in a number of products for consumption by humans as well as animals, such as companion animals (dogs, cats) as well as livestock (bovine, equine, ovine, caprine, or porcine animals,

as well as poultry). For example, a pharmaceutical composition comprises purified 2'-FL and a pharmaceutically-acceptable excipient that is suitable for oral administration. Large quantities of 2'-FL are produced in bacterial hosts, e.g., an *E. coli* bacterium comprising an exogenous α (1,2) fucosyltransferase gene.

[0023] An *E. coli* bacterium comprising an enhanced cytoplasmic pool of lactose and GDP-fucose is useful in such production systems. Endogenous *E. coli* metabolic pathways and genes are manipulated in ways that result in the generation of increased cytoplasmic concentrations of lactose and/or GDP-fucose, as compared to levels found in wild type *E. coli.* For example, the bacteria contain at least 10%, 20%, 50%, or 2X, 5X, 10X or more of the levels compared to a corresponding wild type bacteria that lacks the genetic modifications described above.

[0024] A method of producing a pharmaceutical composition comprising a purified human milk oligosaccharide (HMOS) is carried out by culturing the bacterium described above, purifying the HMOS produced by the bacterium, and combining the HMOS with an excipient or carrier to yield a dietary supplement for oral administration. These compositions are useful in methods of preventing or treating enteric and/or respiratory diseases in infants and adults. Accordingly, the compositions are administered to a subject suffering from or at risk of developing such a disease.

[0025] The invention also provides methods for increasing the intracellular concentration of lactose in *E. coli,* for cells grown in the presence of lactose, by using manipulations of endogenous *E. coli* genes involved in lactose import, export, and catabolism. In particular, described herein are methods of increasing intracellular lactose levels in *E. coli* genetically engineered to produce a human milk oligosaccharide by simultaneous deletion of the endogenous β-galactosidase gene (*lacZ*) and the lactose operon repressor gene (*lacI*). During construction of this deletion, the *lacIq* promoter is placed immediately upstream of (contiguous with) the lactose permease gene, *lacY*, i.e., the sequence of the *lacIq* promoter is directly upstream and adjacent to the start of the sequence encoding the *lacY* gene, such that the *lacY* gene is under transcriptional regulation by the lacIq promoter. The modified strain maintains its ability to transport lactose from the culture medium (via LacY), but is deleted for the wild-type chromosomal copy of the *lacZ* (encoding β-galactosidase) gene responsible for lactose catabolism. Thus, an intracellular lactose pool is created when the modified strain is cultured in the presence of exogenous lactose. Another method for increasing the intracellular concentration of lactose in *E. coli* involves deletion of the *lacA* gene. The *lacA* mutation prevents the formation of intracellular acetyl-lactose, which not only removes this molecule as a contaminant from subsequent purifications, but also eliminates *E.coli*'s ability to *export* excess lactose from its cytoplasm (Danchin A. Cells need safety valves. Bioessays 2009, Jul;31(7):769-73.), thus greatly facilitating purposeful manipulations of the *E.coli* intracellular lactose pool.

[0026] The invention also provides methods for increasing intracellular levels of GDP-fucose in *Escherichia coli* by manipulating the organism's endogenous colanic acid biosynthesis pathway. This increase is achieved through a number of genetic modifications of endogenous *E. coli* genes involved either directly in colanic acid precursor biosynthesis, or in overall control of the colanic acid synthetic regulon. In particular, described herein are methods of increasing intracellular GDP-fucose levels in *E. coli* genetically engineered to produce a human milk oligosaccharide by deletion of the *wcaJ* gene, encoding the UDP-glucose lipid carrier transferase. In a *wcaJ* null background, GDP-fucose accumulates in the *E. coli* cytoplasm.

[0027] In one aspect, the human milk oligosaccharide produced by engineered bacteria comprising an exogenous nucleic acid molecule encoding an α(1,2) fucosyltransferase is 2'- FL (2'-fucosyllactose). Preferably the α (1,2) fucosyltransferase utilized is any α (1,2) fucosyltransferase capable of using lactose as the sugar acceptor substrate for 2'-FL synthesis. Preferably, the exogenous α (1,2) fucosyltransferase gene comprises at least 10% identity at the amino acid level and less than about 40% to *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (FutC).

[0028] The invention also provides compositions comprising *E. coli* genetically engineered to produce the human milk tetrasaccharide lactodifucotetraose (LDFT). The *E. coli* in this instance comprise an exogenous nucleic acid molecule encoding an α (1,2) fucosyltransferase that also possesses α (1,3) fucosyltransferase activity.

[0029] The invention provides a method of treating, preventing, or reducing the risk of infection in a subject comprising administering to said subject a composition comprising a purified recombinant human milk oligosaccharide, wherein the HMOS binds to a pathogen and wherein the subject is infected with or at risk of infection with the pathogen. In one aspect, the infection is caused by a Norwalk-like virus or *Campylobacter jejuni.* The subject is preferably a mammal in need of such treatment. The mammal is, e.g., any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a cow, a horse, or a pig. In a preferred embodiment, the mammal is a human. For example, the compositions are formulated into animal feed (e.g., pellets, kibble, mash) or animal food supplements for companion animals, e.g., dogs or cats, as well as livestock or animals grown for food consumption, e.g., cattle, sheep, pigs, chickens, and goats. Preferably, the purified HMOS is formulated into a powder (e.g., infant formula powder or adult nutritional supplement powder, each of which is mixed with a liquid such as water or juice prior to consumption) or in the form of tablets, capsules or pastes or is incorporated as a component in dairy products such as milk, cream, cheese, yogurt or kefir, or as a component in any beverage, or combined in a preparation containing live microbial cultures intended to serve as probiotics, or in prebiotic preparations to enhance the growth of beneficial microorganisms either *in vitro* or *in vivo.*

[0030] Polynucleotides, polypeptides, and oligosaccharides of the invention are purified and/or isolated. Purified defines a degree of sterility that is safe for administration to a human subject, e.g., lacking infectious or toxic agents.

Specifically, as used herein, an "isolated" or "purified" nucleic acid molecule, polynucleotide, polypeptide, protein or oligosaccharide, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. For example, purified HMOS compositions are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity is measured by any appropriate standard method, for example, by column chromatography, thin layer chromatography, or high-performance liquid chromatography (HPLC) analysis. For example, a "purified protein" refers to a protein that has been separated from other proteins, lipids, and nucleic acids with which it is naturally associated. Preferably, the protein constitutes at least 10, 20, 50, 70, 80, 90, 95, 99-100% by dry weight of the purified preparation.

[0031] Similarly, by "substantially pure" is meant an oligosaccharide that has been separated from the components that naturally accompany it. Typically, the oligosaccharide is substantially pure when it is at least 60%, 70%, 80%, 90%, 95%, or even 99%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated.

[0032] By "isolated nucleic acid" is meant a nucleic acid that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term covers, for example: (a) a DNA which is part of a naturally occurring genomic DNA molecule, but is not flanked by both of the nucleic acid sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner, such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Isolated nucleic acid molecules according to the present invention further include molecules produced synthetically, as well as any nucleic acids that have been altered chemically and/or that have modified backbones.

[0033] A "heterologous promoter" is a promoter which is different from the promoter to which a gene or nucleic acid sequence is operably linked in nature.

[0034] The term "overexpress" or "overexpression" refers to a situation in which more factor is expressed by a genetically-altered cell than would be, under the same conditions, by a wild type cell. Similarly, if an unaltered cell does not express a factor that it is genetically altered to produce, the term "express" (as distinguished from "overexpress") is used indicating the wild type cell did not express the factor at all prior to genetic manipulation.

[0035] The terms "treating" and "treatment" as used herein refer to the administration of an agent or formulation to a clinically symptomatic individual afflicted with an adverse condition, disorder, or disease, so as to effect a reduction in severity and/or frequency of symptoms, eliminate the symptoms and/or their underlying cause, and/or facilitate improvement or remediation of damage. The terms "preventing" and "prevention" refer to the administration of an agent or composition to a clinically asymptomatic individual who is susceptible to a particular adverse condition, disorder, or disease, and thus relates to the prevention of the occurrence of symptoms and/or their underlying cause.

[0036] By the terms "effective amount" and "therapeutically effective amount" of a formulation or formulation component is meant a nontoxic but sufficient amount of the formulation or component to provide the desired effect.

[0037] The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention.

[0038] The host organism used to express the non-FutC lactose-accepting fucosyltransferase gene product is typically the enterobacterium *Escherichia coli* K12 (*E. coli*). *E. coli* K-12 is not considered a human or animal pathogen nor is it toxicogenic. E. coli K-12 is a standard production strain of bacteria and is noted for its safety due to its poor ability to colonize the colon and establish infections (see, e.g., epa.gov/oppt/biotech/pubs/fra/fra004.htm). However, a variety of bacterial species may be used in the oligosaccharide biosynthesis methods, e.g., *Erwinia herbicola* (*Pantoea agglomerans*), *Citrobacter freundii*, *Pantoea citrea*, *Pectobacterium carotovorum*, *or Xanthomonas campestris*. Bacteria of the genus *Bacillus* may also be used, including *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus thermophilus*, *Bacillus laterosporus*, *Bacillus megaterium*, *Bacillus mycoides*, *Bacillus pumilus*, *Bacillus lentus*, *Bacillus cereus*, *and Bacillus circulans*. Similarly, bacteria of the genera *Lactobacillus* and *Lactococcus* may be modified using the methods of this invention, including but not limited to *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus, Lactobacillus crispatus, Lactobacillus gasseri*, *Lactobacillus casei*, *Lactobacillus reuteri*, *Lactobacillus jensenii*, *and Lactococcus lactis. Streptococcus thermophiles and Proprionibacterium freudenreichii* are also suitable bacterial species for the invention described herein. Also included as part of this invention are strains, modified as described here, from the genera *Enterococcus* (e.g., *Enterococcus faecium* and *Enterococcus thermophiles*), *Bifidobacterium* (e.g., *Bifidobacterium longum*, *Bifidobacterium infantis*, and *Bifidobacterium bifdum*), *Sporolactobacillus* spp., *Micromomospora* spp.,

Micrococcus spp., *Rhodococcus* spp., and *Pseudomonas* (e.g., *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*). Bacteria comprising the characteristics described herein are cultured in the presence of lactose, and a fucosylated oligosaccharide is retrieved, either from the bacterium itself or from a culture supernatant of the bacterium. The fucosylated oligosaccharide is purified for use in therapeutic or nutritional products, or the bacteria are used directly in such products.

[0039] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All published foreign patents and patent applications cited herein are incorporated herein by reference. Genbank and NCBI submissions indicated by accession number cited herein are incorporated herein by reference. All other published references, documents, manuscripts and scientific literature cited herein are incorporated herein by reference. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040]

Figure 1 is a schematic illustration showing the synthetic pathway of the fucosyl oligosaccharides of human milk. *Se* and *Le* indicate synthesis by fucosyltransferases of the secretor and Lewis genes, respectively. The abbreviated biochemical name [with alternate biochemical structure in brackets] is given (histo-blood group antigen analog in parentheses).

Figure 2 is a schematic demonstrating metabolic pathways and the changes introduced into them to engineer 2'-fucosyllactose (2'-FL) synthesis in *Escherichia coli* (*E. coli*).

Figure 3 is a series of photographs showing thin layer chromatography analysis of 2'-FL produced in *E. coli* strains by candidate α (1,2) fucosyltransferases. Figure 3A shows significant production of 2'-FL by WbgL. Figure 3B shows significant production of 2'-FL by FutL. Figure 3C shows significant production of 2'-FL by FutN.

Figure 4 is a chart and a photograph of thin layer chromatography analysis showing that fucosidase digestion confirms synthesis of bona fide 2'-FL by WbgL. Oligosaccharides produced by an *E. coli* strain expressing *wbgL* were isolated and subjected to overnight digestion with different fucosidases. Reaction products were analyzed by TLC. The production of fucose and lactose by treatment with α(1,2) fucosidase is illustrated in lane 2.

Figure 5 is a diagram of plasmid pG171.

Figure 6 is a diagram of a P_{laclq} *lacY*$^{+}$ chromosomal construct.

Figure 7 is a diagram of the chromosomal deletion of *wcaJ*.

Figure 8 is a diagram of the *kan, lacZ*$^{+}$ insertion into the *lon* locus.

Figure 9 is a diagram of plasmid pG204.

Figure 10 is a diagram of plasmid pG216.

Figure 11 is a diagram of plasmid pG217.

DETAILED DESCRIPTION OF THE INVENTION

[0041] While some studies suggest that human milk glycans could be used as antimicrobial anti-adhesion agents, the difficulty and expense of producing adequate quantities of these agents of a quality suitable for human consumption has limited their full-scale testing and perceived utility. What has been needed is a suitable method for producing the appropriate glycans in sufficient quantities at reasonable cost. Prior to the invention described herein, there were attempts to use several distinct synthetic approaches for glycan synthesis. Some chemical approaches can synthesize oligosaccharides (Flowers, H. M. Methods Enzymol 50, 93-121 (1978); Seeberger, P. H. Chem Commun (Camb) 1115-1121 (2003)), but reactants for these methods are expensive and potentially toxic (Koeller, K. M. & Wong, C. H. Chem Rev 100, 4465-4494 (2000)). Enzymes expressed from engineered organisms (Albermann, C., Piepersberg, W. & Wehmeier, U. F. Carbohydr Res 334, 97-103 (2001); Bettler, E., Samain, E., Chazalet, V., Bosso, C., et al. Glycoconj J 16, 205-212 (1999); Johnson, K. F. Glycoconj J 16, 141-146 (1999); Palcic, M. M. Curr Opin Biotechnol 10, 616-624 (1999); Wymer, N. & Toone, E. J. Curr Opin Chem Biol 4, 110-119 (2000)) provide a precise and efficient synthesis (Palcic, M. M. Curr Opin Biotechnol 10, 616-624 (1999)); Crout, D. H. & Vic, G. Curr Opin Chem Biol 2, 98-111 (1998)), but the high cost of the reactants, especially the sugar nucleotides, limits their utility for low-cost, large-scale production. Microbes have been genetically engineered to express the glycosyltransferases needed to synthesize oligosaccharides from the bacteria's innate pool of nucleotide sugars (Endo, T., Koizumi, S., Tabata, K., Kakita, S. & Ozaki, A. Carbohydr Res 330, 439-443 (2001); Endo, T., Koizumi, S., Tabata, K. & Ozaki, A. Appl Microbiol Biotechnol 53, 257-261 (2000); Endo, T.

& Koizumi, S. Curr Opin Struct Biol 10, 536-541 (2000); Endo, T., Koizumi, S., Tabata, K., Kakita, S. & Ozaki, A. Carbohydr Res 316, 179-183 (1999); Koizumi, S., Endo, T., Tabata, K. & Ozaki, A. Nat Biotechnol 16, 847-850 (1998)). However, prior to the invention described herein, there was a growing need to identify and characterize additional glycosyltransferases that are useful for the synthesis of HMOS in metabolically engineered bacterial hosts.

[0042] Not all α(1,2)fucosyltransferases can utilize lactose as an acceptor sugar. A desired enzyme utilizes GDP-fucose as a donor, and lactose is the acceptor for that donor. A method of identifying novel α(1,2)fucosyltransferase enzymes capable of utilizing lactose as an acceptor was carried out using the following steps:: 1) performing a computational search of sequence databases to define a broad group of simple sequence homologs of any known, lactose-utilizing α(1,2)fucosyltransferase; 2) using the list of homologs from step 1 to derive a search profile containing common sequence and/or structural motifs shared by the members of the broad group, e.g. by using computer programs such as MEME (Multiple Em for Motif Elicitation available at http://meme.sdsc.edu/meme/cgi-bin/meme.cgi) or PSI-BLAST (Position-Specific Iterated BLAST available at ncbi.nlm.nih.gov/blast with additional information at cnx.org/content/ml 1040/latest/); 3) searching sequence databases (e.g., using computer programs such as PSI-BLAST, or MAST (Motif Alignment Search Tool available at http://meme.sdsc.edu/meme/cgi-bin/mast.cgi);, using this derived search profile as query, and identifying "candidate sequences" whose simple sequence homology to the original lactose-accepting α(1,2)fucosyltransferase is 40% or less; 4) scanning the scientific literature and developing a list of "candidate organisms" known to express α(1,2)fucosyl-glycans; 5) selecting only those "candidate sequences" that are derived from "candidate organisms" to generate a list of "candidate lactose-utilizing enzymes"; and 6) expressing each "candidate lactose-utilizing enzyme" and testing for lactose-utilizing α(1,2)fucosyltransferase activity.

[0043] The MEME suite of sequence analysis tools (meme.sdsc.edu/meme/cgi-bin/meme.cgi) can also be used as an alternative to PSI-BLAST. Sequence motifs are discovered using the program "MEME". These motifs can then be used to search sequence databases using the program "MAST". The BLAST and PHI-BLAST search algorithms are other well known alternatives.

[0044] To test for lactose-utilizing activity, the production of 2'-FL is evaluated in a host organism that expresses the candidate enzyme and which contains both cytoplasmic GDP-fucose and lactose pools. The production of 2'-FL indicates that the candidate enzyme-encoding sequence functions as a lactose-utilizing α(1,2)fucosyltransferase.

[0045] To find enzymes with similarity to FutC, entire amino acid of FutC was used as a query in PSI-BLAST. The results of the lactose-utilizing α(1,2)fucosyltransferase identification method of this invention are surprising, because the % identity of several of the lactose-utilizing α(1,2)fucosyltransferases identified are less than 40% of the reference FutC sequence. Another most surprising aspect of the study is that 8 of the 10 candidates tested were able to utilize lactose as an acceptor, 3 of which did so at levels very close to the "gold-standard" enzyme FutC. This was a higher "hit rate" was anticipated. While 6 out of 10 of the candidate enzymes are found in bacteria that incorporate α(1,2)fucose into their LPS structure, the oligosaccharides to which the fucose is attached are very different than the lactose each candidate enzyme is being asked to utilize in the query. Moreover, it was surprising that both WblA and WbgN could utilize lactose as an acceptor, because both of these enzymes are found in bacteria that do not incorporate fucose into their LPS structure. Rather, they utilize a related sugar called colitose.

Human Milk Glycans

[0046] Human milk contains a diverse and abundant set of neutral and acidic oligosaccharides (Kunz, C., Rudloff, S., Baier, W., Klein, N., and Strobel, S. (2000). Annu Rev Nutr 20, 699-722; Bode, L. (2006). J Nutr 136, 2127-130). More than 130 different complex oligosaccharides have been identified in human milk, and their structural diversity and abundance is unique to humans. Although these molecules may not be utilized directly by infants for nutrition, they nevertheless serve critical roles in the establishment of a healthy gut microbiome (Marcobal, A., Barboza, M., Froehlich, J. W., Block, D. E., et al. J Agric Food Chem 58, 5334-5340 (2010)), in the prevention of disease (Newburg, D. S., Ruiz-Palacios, G. M. & Morrow, A. L. Annu Rev Nutr 25, 37-58 (2005)), and in immune function (Newburg, D. S. & Walker, W. A. Pediatr Res 61, 2-8 (2007)). Despite millions of years of exposure to human milk oligosaccharides (HMOS), pathogens have yet to develop ways to circumvent the ability of HMOS to prevent adhesion to target cells and to inhibit infection. The ability to utilize HMOS as pathogen adherence inhibitors promises to address the current crisis of burgeoning antibiotic resistance. Human milk oligosaccharides produced by biosynthesis represent the lead compounds of a novel class of therapeutics against some of the most intractable scourges of society.

[0047] One alternative strategy for efficient, industrial-scale synthesis of HMOS is the metabolic engineering of bacteria. This approach involves the construction of microbial strains overexpressing heterologous glycosyltransferases, membrane transporters for the import of precursor sugars into the bacterial cytosol, and possessing enhanced pools of regenerating nucleotide sugars for use as biosynthetic precursors (Dumon, C., Samain, E., and Priem, B. (2004). Biotechnol Prog 20, 412-19; Ruffing, A., and Chen, R.R. (2006). Microb Cell Fact 5, 25). A key aspect of this approach is the heterologous glycosyltransferase selected for overexpression in the microbial host. The choice of glycosyltransferase can significantly affect the final yield of the desired synthesized oligosaccharide, given that enzymes can vary greatly

in terms of kinetics, substrate specificity, affinity for donor and acceptor molecules, stability and solubility. A few glycosyltransferases derived from different bacterial species have been identified and characterized in terms of their ability to catalyze the biosynthesis of HMOS in *E. coli* host strains (Dumon, C., Bosso, C., Utille, J.P., Heyraud, A., and Samain, E. (2006). Chembiochem 7, 359-365; Dumon, C., Samain, E., and Priem, B. (2004). Biotechnol Prog 20, 412-19; Li, M., Liu, X.W., Shao, J., Shen, J., Jia, Q., Yi, W., Song, J.K., Woodward, R., Chow, C.S., and Wang, P.G. (2008). Biochemistry 47, 378-387). The identification of additional glycosyltransferases with faster kinetics, greater affinity for nucleotide sugar donors and/or acceptor molecules, or greater stability within the bacterial host significantly improves the yields of therapeutically useful HMOS. Prior to the invention described herein, chemical syntheses of HMOS were possible, but were limited by stereo-specificity issues, precursor availability, product impurities, and high overall cost (Flowers, H. M. Methods Enzymol 50, 93-121 (1978); Seeberger, P. H. Chem Commun (Camb) 1115-1121 (2003); Koeller, K. M. & Wong, C. H. Chem Rev 100, 4465-4494 (2000)). The invention overcomes the shortcomings of these previous attempts by providing new strategies to inexpensively manufacture large quantities of human milk oligosaccharides (HMOS) for use as dietary supplements. Advantages include efficient expression of the enzyme, improved stability and/or solubility of the gene product (2'-FL) and reduced toxicity to the host organism. For example, α(1,2) fucosyltransferases derived from *E.coli* strains (e.g. WbgL) are more stable and are expressed at higher levels within *E.coli* production hosts strains compared to FutC. In another example, highly active fucosyltransferase (futN) is derived from a commensal microbe (*Bacteroides*) rather than a pathogen. Since many engineered production strains use fucosyltransferase genes obtained from pathogens, safety and/or increased consumer acceptance are added advantages of this sequence/enzyme.

[0048] As described in detail below, *E. coli* (or other bacteria) is engineered to produce 2'-FL in commercially viable levels. For example, yields are >5 grams/liter in a bacterial fermentation process.

Role of Human milk glycans in infectious disease

[0049] Human milk glycans, which comprise both unbound oligosaccharides and their glycoconjugates, play a significant role in the protection and development of the infant gastrointestinal (GI) tract. Neutral fucosylated oligosaccharides, including 2'-fucosyllactose (2'-FL), protect infants against several important pathogens. Milk oligosaccharides found in various mammals differ greatly, and the composition in humans is unique (Hamosh M., 2001 Pediatr Clin North Am, 48:69-86; Newburg D.S., 2001 Adv Exp Med Biol, 501:3-10). Moreover, glycan levels in human milk change throughout lactation and also vary widely among individuals (Morrow A.L. et al., 2004 J Pediatr, 145:297-303; Chaturvedi P et al., 2001 Glycobiology, 11:365-372). Approximately 200 distinct human milk oligosaccharides have been identified and combinations of simple epitopes are responsible for this diversity (Newburg D.S., 1999 Curr_Med Chem, 6:117-127; Ninonuevo M. et al., 2006 J Agric Food Chem, 54:7471-74801).

[0050] Human milk oligosaccharides are composed of 5 monosaccharides: D-glucose (Glc), D-galactose (Gal), N-acetylglucosamine (GlcNAc), L-fucose (Fuc), and sialic acid (N-acetyl neuraminic acid, Neu5Ac, NANA). Human milk oligosaccharides are usually divided into two groups according to their chemical structures: neutral compounds containing Glc, Gal, GlcNAc, and Fuc, linked to a lactose (Galβ1-4Glc) core, and acidic compounds including the same sugars, and often the same core structures, plus NANA (Charlwood J. et al., 1999 Anal Biochem, 273:261-277; Martin-Sosa et al., 2003 J Dairy Sci, 86:52-59; Parkkinen J. and Finne J., 1987 Methods Enzymol, 138:289-300; Shen Z. et al., 2001 J Chromatogr A, 921:315-321).

[0051] Approximately 70-80% of oligosaccharides in human milk are fucosylated, and their synthetic pathways are believed to proceed as shown in Figure 1 (Type I and Type II pathways begin with different precursor molecules). A smaller proportion of the oligosaccharides are sialylated or both fucosylated and sialylated, but their synthetic pathways are not fully defined. Understanding of the acidic (sialylated) oligosaccharides is limited in part by the ability to measure these compounds. Sensitive and reproducible methods for the analysis of both neutral and acidic oligosaccharides have been designed. Human milk oligosaccharides as a class survive transit through the intestine of infants very efficiently, being essentially indigestible (Chaturvedi, P., Warren, C. D., Buescher, C. R., Pickering, L. K. & Newburg, D. S. Adv Exp Med Biol 501, 315-323 (2001)).

Human milk glycans inhibit binding of enteropathogens to their receptors

[0052] Human milk glycans have structural homology to cell receptors for enteropathogens and function as receptor decoys. For example, pathogenic strains of *Campylobacter* bind specifically to glycans containing H-2, i.e., 2'-fucosyl-*N*-acetyllactosamine or 2'-fucosyllactose (2'FL); *Campylobacter* binding and infectivity are inhibited by 2'-FL and other glycans containing this H-2 epitope. Similarly, some diarrheagenic *E. coli* pathogens are strongly inhibited *in vivo* by human milk oligosaccharides containing 2-linked fucose moieties. Several major strains of human caliciviruses, especially the noroviruses, also bind to 2-linked fucosylated glycans, and this binding is inhibited by human milk 2-linked fucosylated glycans. Consumption of human milk that has high levels of these 2-linked fucosyloligosaccharides was associated with lower risk of norovirus, *Campylobacter*, ST of *E. coli*-associated diarrhea, and moderate-to-severe diarrhea of all causes

in a Mexican cohort of breastfeeding children (Newburg D.S. et al., 2004 Glycobiology, 14:253-263; Newburg D.S. et al., 1998 Lancet, 351:1160-1164). Several pathogens utilize sialylated glycans as their host receptors, such as influenza (Couceiro, J. N., Paulson, J. C. & Baum, L. G. Virus Res 29, 155-165 (1993)), parainfluenza (Amonsen, M., Smith, D. F., Cummings, R. D. & Air, G. M. J Virol 81, 8341-8345 (2007), and rotoviruses (Kuhlenschmidt, T. B., Hanafin, W. P., Gelberg, H. B. & Kuhlenschmidt, M. S. Adv Exp Med Biol 473, 309-317 (1999)). The sialyl-Lewis X epitope is used by *Helicobacter pylori* (Mahdavi, J., Sondén, B., Hurtig, M., Olfat, F. O., et al. Science 297, 573-578 (2002)), *Pseudomonas aeruginosa* (Scharfman, A., Delmotte, P., Beau, J., Lamblin, G., et al. Glycoconj J 17, 735-740 (2000)), and some strains of noroviruses (Rydell, G. E., Nilsson, J., Rodriguez-Diaz, J., Ruvoën-Clouet, N., et al. Glycobiology 19, 309-320 (2009)).

Engineering of *E. coli* to produce human milk oligosaccharide 2'-FL

[0053] Described herein is a gene screening approach, which was used to identify new α (1,2) fucosyltransferases (α (1,2) FTs) for the synthesis of fucosyl-linked oligosaccharides in metabolically engineered *E. coli.* Of particular interest are α (1,2) FTs that are capable of the synthesis of the HMOS 2'-fucosyllactose (2'-FL). 2'-FL is the most abundant fucosylated oligosaccharide present in human milk, and this oligosaccharide provides protection to newborn infants against infectious diarrhea caused by bacterial pathogens such as *Campylobacter jejuni* (Ruiz-Palacios, G.M., et al. (2003). J Biol Chem 278, 14112-120; Morrow, A.L. et al. (2004). J Pediatr 145, 297-303; Newburg, D.S. et al. (2004). Glycobiology 14, 253-263).

[0054] The synthetic pathway of the fucosyl oligosaccharides of human milk is illustrated in Figure 1. Structurally, 2'-FL consists of a fucose molecule α 1,2 linked to the galactose portion of lactose (Fucα1-2Galβ1-4Glc). An α (1,2) FT from *H. pylori* strain 26695 termed FutC has been utilized to catalyze the synthesis of 2'-FL in metabolically engineered *E. coli* (Drouillard, S. et al. (2006). Angew Chem Int Ed Engl 45, 1778-780). Therefore, the amino acid sequence of FutC was used as a query in the search algorithm PSI-BLAST (Position Specific Iterated Basic Local Alignment Search Tool) to identify candidate novel α (1,2) FTs for the production of 2'-FL in *E. coli.* Using PSI-BLAST, a list of closely related protein sequences is created based on the query sequence. The algorithm then generates a profile sequence, which summarizes significant motifs present in these sequences. This profile is then used as a new query to identify a larger group of candidate sequences, and the process is iterated to generate an even larger group of candidates.

[0055] The FutC amino acid sequence was used as a query for 2 iterations of the PSI-BLAST search algorithm. This search yielded a group of 277 candidates with similarity to FutC, some of which were more closely related (shared amino acid identity greater than 25%) as well as a group that was more distantly related to FutC (shared amino acid identity less than 25%). Of the more closely related group, the predicted α(1,2) FTs from bacterial species that incorporate fucose into the O-antigen of their lipopolysaccharide (LPS) or into the polysaccharide subunits that compose the cell surface capsule were analyzed. α(1,2) FTs from these types of organisms are more likely to utilize fucose as a substrate, given the presence of fucose in their surface carbohydrate structures. α(1,2) FTs from known enteric bacterial species, either commensals or pathogens were also analyzed. Such organisms sometimes display carbohydrate structures on their cell-surface that contain fucose and mimic various 2'-fucosyl containing Lewis antigen structures found in higher organisms (Appelmelk, B.J. et al. (1998). Infect Immun 66, 70-76; Coyne, M.J. et al. (2005). Science 307, 1778-781). Candidate α(1,2) FTs from these types of organisms are more likely to utilize fucose as a substrate and also to catalyze the linkage of fucose to useful acceptor oligosaccharides.

[0056] Ten α(1,2) FTs with greater than 25% homology at the amino acid level to FutC identified from the screen were analyzed (Table 1).

Table 1. Summary of candidate α (1,2) fucosyltransferases tested for their ability to promote 2'-FL in engineered *E. coli* strains. The activity of each candidate was compared to FutC and described semi-quantitatively using the "+" symbol in the last column, where FutC is assessed the highest activity with 4 "+" symbols.

Gene Name	Accession No. (NCBI)	Organism	% Identity w/ futC	Fucose in LPS of capsule?	2'-FL. Synthesis
futC	NP_206893 NP_206894	*H.pylori* 26685	-	Yes	+++
wblA	BAA33632	*V.cholerae* O22	28%	No	+
wbgL	ADNA3847	*E.coli* 0125	25%	Yes	+++
futD	ZP_04580654	*H. bilis* ATCC 437873	39%	Yes	+
futE	*ZP*_07805473	*H. cinoedi* CCUG 18818	44%	Unknown	-

(continued)

Gene Name	Accession No. (NCBI)	Organism	% Identity w/ futC	Fucose in LPS of capsule?	2'-FL. Synthesis
futL	YP_003517185	*B. mustelae* ATCC 43772	70%	Yes	+++
futN	YP_001300461	*B. vulgatus* ATCC 8482	27%	Unknown	++
futO	ZP_02065239	*B. ovalus* ATCC 8483	27%	Unknown	-
wbgN	YP_003900093	*E. coli* 095:M7	28%	No	+
bft1	CAM09389	*B. fragilis* 9343	34%	Yes	-
bft3/*wcfB*	CAH06753	*B. fragilis* 9343	28%	Yes	+

[0057] The amino acid sequence of *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (FutC) is set forth below (SEQ ID NO: 2; GenBank Accession Number NP_206893 and NP_206894 (GI:15644723 and 15644724), incorporated herein by reference).

```
  1 mafkvvqicg glgnqmfqya fakslqkhln tpvllditsf dwsnrkmqle lfpidlpyas

 61 akeiaiakmq hlpklvrdtl kcmgfdrvsq eivfeyepgl lkpsrltyfy gyfqdpryfd
121 aisplikqtf tlpppengnn kkkeeeyhrk lalilaakns vfvhvrrgdy vgigcqlgid
181 yqkkaleyia krvpnmelfv fcedlkftqn ldlgypfmdm ttrdkeeeay wdmllmqsck
241 hgiianstys wwaaylinnp ekiiigpkhw lfghenilck ewvkieshfe vkskkyna
```

[0058] The amino acid sequence of *Vibrio cholera* 022 WbIA is set forth below (SEQ ID NO: 3; GenBank Accession Number BAA33632 (GI:3721682), incorporated herein by reference).

```
  1 mivmkisggl gnqlfqyavg raiaiqygvp lkldvsaykn yklhngyrld qfninadian
 61 edeifhlkgs snrlsrilrr lgwlkkntyy aekqrtiydv svfmqapryl dgywqneqyf
121 sqiravllqe lwpnqplsin aqahqikiqq thavsihvrr gdylnhpeig vldidyykra
181 vdyikekiea pvffvfsndv awckdnfnfi dspvfiedtq teiddlmlmc qcqhnivans
241 sfswwaawln snvdkiviap ktwmaenpkg ykwvpdswre i
```

[0059] The amino acid sequence of *Escherichia coli* 0126 WbgL is set forth below (SEQ ID NO: 4; GenBank Accession Number ADN43847 (GI:307340785), incorporated herein by reference).

```
  1 msiirlqggl gnqlfqfsfg yalskingtp lyfdishyae nddhggyrln nlqipeeylq
 61 yytpkinniy kflvrgsrly peiflflgfc nefhaygydf eyiaqkwksk kyigywqseh
121 ffhkhildlk effipknvse qanllaakil esqsslsihi rrgdyiknkt atlthgvcsl
181 eyykkalnki rdlamirdvf ifsddifwck enietllskk yniyysedls qeedlwlmsl
241 anhhiianss fswwgaylgt sasqiviypt pwyditpknt yipivnhwin vdkhssc
```

[0060] The amino acid sequence of *Helicobacter bilis* ATCC 437879 FutD is set forth below (SEQ ID NO: 5; GenBank Accession Number ZP_04580654 (GI: 237750174), incorporated herein by reference).

```
  1 mgdykivelt cglgnqmfqy afakalqkhl qvpvlldktw ydtqdnstqf sldifnvdle
 61  yatntqieka karvsklpgl lrkmfglkkh niaysqsfdf hdeyllpndf tyfsgffqna
121 kylkgleqel ksifyydsnn fsnfgkqrle lilqaknsif ihirrgdyck igwelgmdyy
181 kraiqyimdr veepkffifg atdmsfteqf qknlglnenn sanlsektit qdnqhedmfl
241 mcyckhaila nssysfwsay lnndannivi aptpwlldnd niicddwiki ssk
```

[0061] The amino acid sequence of *Helicobacter cinaedi* CCUG 18818 alpha-1,2-fucosyltransferase (FutE) is set forth below (SEQ ID NO: 6; GenBank Accession Number ZP_07805473 (GI:313143280), incorporated herein by reference).

```
  1 mlfpfkfiyn rlrykairli rrrasyrpfy efyahivwge egvvndrimk hyressfkpy

 61 afpyginmsf vysndvyall kddfrlkipl rydnamlkkq iqntdksvfl hirrgdylqs
121 eglyvvlgvt yyqkaleilk skitnphifv fsndmcwcke ylmryvdfsg ctidfiegnt
181 egnaveemel mrscqhaiia nstfswwaay lienpdkivi mpkeylndss rflpkqflal
241 knwflvdhiw gsvelan
```

[0062] The amino acid sequence of *Helicobacter mustelae* 12198 (ATCC 43772) alpha-1,2-fucosyltransferase (FutL) is set forth below (SEQ ID NO: 7; GenBank Accession Number YP_003517185 (GI:291277413), incorporated herein by reference).

```
  1 mdfkivqvhg glgnqmfqya fakslqthln ipvlldttwf dygnrelglh lfpidlqcas
 61 aqqiaaahmq nlprlvrgal rrmglgrvsk eivfeympel fepsriayfh gyfqdpryfe
121 displikqtf tlphptehae qysrklsqil aaknsvfvhi rrgdymrlgw qldisyqlra
181 iaymakrvqn lelflfcedl efvqnldlgy pfvdmttrdg aahwdmmlmq sckhgiitns
241 tyswwaayli knpekiiigp shwiygneni lckdwvkies qfetks
```

[0063] One α(1,2)fucosyltransferase identified through the screen that possessed comparable enzymatic activity relative to FutC was termed FutL. FutL was found to direct the synthesis of 2'-FL at ~75% the level of FutC in the metabolically engineered E. coli production strain (Table 1 and Figure 3). In addition, the data indicated that FutL is significantly less efficient at promoting the synthesis of LDFT, a byproduct that was observed with other α(1,2)FTs. Therefore, FutL offers advantages over the others, e.g., the ability to robustly produce 2'-FL without the concern of concurrently producing other undesirable contaminating oligosaccharides. FutL is derived from *Helicobacter mustelae* and is 70% identical to FutC at the amino acid level.

[0064] The amino acid sequence of *Bacteroides vulgatus* ATCC 8482 glycosyl transferase family protein (FutN) is set forth below (SEQ ID NO: 8; GenBank Accession Number YP_001300461 (GI:150005717), incorporated herein by reference).

```
  1 mrlikvtggl gnqmfiyafy lrmkkyypkv ridlsdmmhy kvhygyemhr vfnlphtefc
 61 inqplkkvie flffkkiyer kqapnslraf ekkyfwplly fkgfyqserf fadikdevre
121 sftfdknkan srslnmleil dkdenavslh irrgdylqpk hwattgsvcq lpyyqnaiae
181 msrrvaspsy yifsddiawv kenlplqnav yidwntdeds wqdmmlmshc khhiicnstf
241 swwgawlnpn mdktvivpsr wfqhseapdi yptgwikvpv s
```

[0065] The amino acid sequence of *Bacteroides ovatus* ATCC 8483 FutO is set forth below (SEQ ID NO: 9; GenBank Accession Number ZP_02065239 (GI: 160884236), incorporated herein by reference).

```
  1 mkivnilggl gnqmfvyamy lalkeahpee eillcrrsyk gyplhngyel erifgveape
 61 aalsqlarva ypffnykswq lmrhflplrk smasgttqip fdysevtrnd nvyydgywqn
121 eknflsirdk vikaftfpef rdeknkalsd klksvktasc hirrgdylkd piygvcnsdy

181 ytraitelnq svnpdmycif sddigwcken fkfligdkev vfvdwnkgqe sfydmqlmsl
241 chyniianss fswwgawlnn nddkvvvape rwmnktlend picdnwkrik ve
```

[0066] The amino acid sequence of *Escherichia coli* O55:H7 (str. CB9615) fucosyltransferase (WbgN) is set forth below (SEQ ID NO: 10; GenBank Accession Number YP_003500093 (GI:291283275), incorporated herein by reference).

```
  1 msivvarlag glgnqmfqya kgyaesvern sslkldlrgy knytlhggfr ldklnidntf
 61 vmskkemcif pnfivraink fpklslcskr feseqyskki ngsmkgsvef igfwqneryf
121 lehkeklrei ftpininlda kelsdvirct nsvsvhirrg dyvsnvealk ihglcteryy
181 idsirylker fnnlvffvfs ddiewckkyk neifsrsddv kfiegntqev dmwlmsnaky
241 hiianssfsw wgawlknydl gitiaptpwf ereelnsfdp cpekwvriek
```

[0067] The amino acid sequence of *Bacteroides fragilis* (NCTC) 9343 alpha-1,2-fucosyltransferase (Bft1) is set forth below (SEQ ID NO: 11, GenBank Accession Number CAH09369 (GI:60494568), incorporated herein by reference).

```
  1 mffrccmkiv qiigglgnqm fqfafylalk ekyvnvkldt ssfgaythng feldkvfhve
 61 ylkasireri klsyqgseiw irvlrkllkr kkteyvepyl cfdenaisls cdkyyigywq
121 sykyftniea airgqfhfsk vlsdknefik kqmqnsnsvs lhvrlgdyvn npaysnicts
181 ayynkainii qskvsepkff vfsddtvwck dhlkipnchi idwnnkeesy wdmclmtyck
241 hniianssfs wwgawlntnp eriviapgkw inddrvqvsd iipsdwicv
```

[0068] The amino acid sequence of *Bacteroides fragilis* (NCTC) 9343 fucosyl transferase (Bft3/WcfB) is set forth below (SEQ ID NO: 12; GenBank Accession Number CAH06753 (GI:60491992), incorporated herein by reference).

```
  1 mlyvilrgrl gnnlfqiata asltqnfifc tvnkdqerqv llykdsffkn ikvmkgvpdg
 61 ipyykepfhe fsripyeegk dliidgyfqs ekyfkrsvvl dlyritdelr kkiwnicgni
121 lekgetvsih vrrgdylklp halpfcgksy yknaiqyige dkifiicsdd idwckknfig
181 kryyfientt plldlyiqsl cthniisnss fswwgawlne nsnkiviapq mwfgisvklg
241 vsdllpvswv rlpnnytlgr ycfalykvve dyllnilrli wkrkknm
```

Homology comparison matrix of fucosyltransferases examined in this study:

	FutC	**WbsJ**	Wbgt	**WblR**	**WbgN**	**Bft1**	**Bft3**	**FutD**	**FutE**	FutC	**FutN**	FutC
FutC	-	30%	28%	28%	28%	34%	28%	39%	44%	70%	27%	27%
WbsJ	30%	-	33%	33%	36%	36%	40%	30%	35%	30%	33%	36%
WbgL	28%	33%	-	33%	37%	32%	39%	31%	32%	25%	32%	33%
WblA	28%	33%	33%	-	36%	37%	33%	31%	38%	29%	31%	35%
WbgN	28%	36%	37%	36%	-	32%	37%	30%	38%	30%	32%	35%
Bft1	34%	36%	32%	37%	32%	-	30%	32%	37%	33%	35%	38%
Bft3	28%	40%	39%	33%	37%	30%	-	30%	33%	29%	34%	35%
FutD	39%	30%	31%	31%	30%	32%	30%	-	34%	40%	28%	31%

(continued)

	FutC	**WbsJ**	Wbgt	**WbIR**	**WbgN**	**Bft1**	**Bft3**	**FutD**	**FutE**	FutC	**FutN**	FutC
FutE	44%	35%	32%	38%	38%	37%	33%	34%	-	33%	33%	36%
FutL	70%	30%	25%	29%	30%	33%	29%	40%	33%	-	30%	28%
FutN	27%	33%	32%	31%	32%	35%	34%	28%	34%	30%	-	37%
FutO	27%	36%	33%	35%	35%	38%	35%	31%	36%	28%	37%	-

[0069] All of these proteins are found in bacteria that interact with the gastrointestinal system of higher organisms. In addition, 6 of the 10 selected incorporate fucose into their cell surface glycans. Such genes were predicted to have the strongest activity in terms of fucosyl-oligosaccharide synthesis. In this group of 10 candidates, 2 enzymes found in bacterial strains that do not incorporate fucose into cell surface glycans (WbIA and WbgN) were also included. It was predicted that these candidates would have little or no fucosyl-oligosaccharide synthesis activity, and therefore might serve as a useful negative control to validate the screening approach.

[0070] Candidate α(1,2) FTs were cloned by standard molecular biological techniques into an expression plasmid. This plasmid utilizes the strong leftwards promoter of bacteriophage λ (termed P_L) to direct expression of the candidate genes (Sanger, F. et al. (1982). J Mol Biol 162, 729-773). The promoter is controllable, e.g., a trp-cI construct is stably integrated the into the E.coli host's genome (at the ampC locus), and control is implemented by adding tryptophan to the growth media. Gradual induction of protein expression is accomplished using a temperature sensitive cI repressor. Another similar control strategy (temperature independent expression system) has been described (Mieschendahl et al., 1986, Bio/Technology 4:802-808)._The plasmid also carries the *E. coli rcsA* gene to up-regulate GDP-fucose synthesis, a critical precursor for the synthesis of fucosyl-linked oligosaccharides. In addition, the plasmid carries a β-lactamase (*bla*) gene for maintaining the plasmid in host strains by ampicillin selection (for convenience in the laboratory) and a native *thyA* (thymidylate synthase) gene as an alternative means of selection in *thyA*$^-$ hosts. Alternative selectable markers include the proBA genes to complement proline auxotrophy (Stein et al., (1984), J Bacteriol 158:2, 696-700 (1984) or purC to complement adenine auxotrophy (Parker, J., (1984), J Bacteriol 157:3, 712-7). To act as plasmid selectable markers each of these genes are first inactivated in the host cell chromosome, then wild type copies of the genes are provided on the plasmid. Alternatively a drug resistance gene may be used on the plasmid, e.g. beta-lactamase (this gene is already on the expression plasmid described above, thereby permitting selection with ampicillin). Ampicilline selection is well known in the art and described in standard manuals such as Maniatis et al., (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring, NY.

[0071] The expression constructs were transformed into a host strain useful for the production of 2'-FL. Biosynthesis of 2'-FL requires the generation of an enhanced cellular pool of both lactose and GDP-fucose (Figure 2). The wild-type *Eschericia coli* K12 prototrophic strain W3110 was selected as the parent background to test the ability of the candidates to catalyze 2'-FL production (Bachmann, B.J. (1972). Bacteriol Rev 36, 525-557). The particular W3110 derivative employed was one that previously had been modified by the introduction (at the *ampC* locus) of a tryptophan-inducible P_{trpB} *cI*+ repressor cassette, generating an *E.coli* strain known as GI724 (LaVallie, E.R. et al. (2000). Methods Enzymol 326, 322-340). Other features of GI724 include *lacIq* and *lacPL8* promoter mutations. *E.coli* strain GI724 affords economical production of recombinant proteins from the phage λ P_L promoter following induction with low levels of exogenous tryptophan (LaVallie, E.R. et al. (1993). Biotechnology (N Y) 11, 187-193; Mieschendahl, et al. (1986). Bio/Technology 4, 802-08). Additional genetic alterations were made to this strain to promote the biosynthesis of 2'-FL. This was achieved in strain GI724 through several manipulations of the chromosome using λ Red recombineering (Court, D.L. et al. (2002). Annu Rev Genet 36, 361-388) and generalized P1 phage transduction.

[0072] First, the ability of the *E. coli* host strain to accumulate intracellular lactose was engineered by simultaneous deletion of the endogenous β-galactosidase gene (*lacZ*) and the lactose operon repressor gene (*lacI*). During construction of this deletion, the *lacIq* promoter was placed immediately upstream of the lactose permease gene, *lacY.* The modified strain maintains its ability to transport lactose from the culture medium (via LacY), but is deleted for the wild-type copy of the *lacZ* (β-galactosidase) gene responsible for lactose catabolism. Therefore, an intracellular lactose pool is created when the modified strain is cultured in the presence of exogenous lactose. A schematic of the P_{lacIq} *lacY*$^+$ chromosomal construct is shown in Figure 6.

[0073] Genomic DNA sequence of the P_{lacIq} *lacY*$^+$ chromosomal construct is set forth below (SEQ ID NO: 13) (the ATG start codon is underlined):

CACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGA
GAGTCAAGTGTAGGCTGGAGCTGCTTCGAAGTTCCTATACTTTCTAGAGAATAGG
AACTTCGGAATAGGAACTTCGGAATAGGAACTAAGGAGGATATTCATATGTACT
ATTTAAAAAACACAAACTTTTGGATGTTCGGTTTATTCTTTTTCTTTTACTTTTTTA
TCATGGGAGCCTACTTCCCGTTTTTCCCGATTTGGCTACATGACATCAACCATATC
AGCAAAAGTGATACGGGTATTATTTTTGCCGCTATTTCTCTGTTCTCGCTATTATT
CCAACCGCTGTTTGGTCTGCTTTCTGACAAACTCGGGCTGCGCAAATACCTGCTG
TGGATTATTACCGGCATGTTAGTGATGTTTGCGCCGTTCTTTATTTTTATCTTCGG
GCCACTGTTACAATACAACATTTTAGTAGGATCGATTGTTGGTGGTATTTATCTA
GGCTTTTGTTTTAACGCCGGTGCGCCAGCAGTAGAGGCATTTATTGAGAAAGTCA
GCCGTCGCAGTAATTTCGAATTTGGTCGCGCGCGGATGTTTGGCTGTGTTGGCTG
GGCGCTGTGTGCCTCGATTGTCGGCATCATGTTCACCATCAATAATCAGTTTGTTT
TCTGGCTGGGCTCTGGCTGTGCACTCATCCTCGCCGTTTTACTCTTTTTCGCCAAA
ACGGATGCGCCCTCTTCTGCCACGGTTGCCAATGCGGTAGGTGCCAACCATTCGG
CATTTAGCCTTAAGCTGGCACTGGAACTGTTCAGACAGCCAAAACTGTGGTTTTT
GTCACTGTATGTTATTGGCGTTTCCTGCACCTACGATGTTTTTGACCAACAGTTTG
CTAATTTCTTTACTTCGTTCTTTGCTACCGGTGAACAGGGTACGCGGGTATTTGGC
TACGTAACGACAATGGGCGAATTACTTAACGCCTCGATTATGTTCTTTGCGCCAC
TGATCATTAATCGCATCGGTGGGAAAAACGCCCTGCTGCTGGCTGGCACTATTAT
GTCTGTACGTATTATTGGCTCATCGTTCGCCACCTCAGCGCTGGAAGTGGTTATTC
TGAAAACGCTGCATATGTTTGAAGTACCGTTCCTGCTGGTGGGCTGCTTTAAATA
TATTACCAGCCAGTTTGAAGTGCGTTTTTCAGCGACGATTTATCTGGTCTGTTTCT
GCTTCTTTAAGCAACTGGCGATGATTTTTATGTCTGTACTGGCGGGCAATATGTAT
GAAAGCATCGGTTTCCAGGGCGCTTATCTGGTGCTGGGTCTGGTGGCGCTGGGCT
TCACCTTAATTTCCGTGTTCACGCTTAGCGGCCCCGGCCCGCTTTCCCTGCTGCGT
CGTCAGGTGAATGAAGTCGCTTAAGCAATCAATGTCGGATGCGGCGCGAGCGCC
TTATCCGACCAACATATCATAACGGAGTGATCGCATTGTAAATTATAAAAATTGC
CTGATACGCTGCGCTTATCAGGCCTACAAGTTCAGCGATCTACATTAGCCGCATC
CGGCATGAACAAAGCGCAGGAACAAGCGTCGCA

[0074] Second, the ability of the host *E. coli* strain to synthesize colanic acid, an extracellular capsular polysaccharide, was eliminated by the deletion of the *wcaJ* gene, encoding the UDP-glucose lipid carrier transferase (Stevenson, G. et al. (1996). J Bacteriol 178, 4885-893). In a *wcaJ* null background GDP-fucose accumulates in the *E. coli* cytoplasm (Dumon, C. et al. (2001). Glycoconj J 18, 465-474). A schematic of the chromosomal deletion of *wcaJ* is shown in Figure 7.

[0075] The sequence of the chromosomal region bearing the *ΔwcaJ*::FRT mutation is set forth below (SEQ ID NO: 14) (the mutation is underlined):

GTTCGGTTATATCAATGTCAAAAACCTCACGCCGCTCAAGCTGGTGATCAACTCC
GGGAACGGCGCAGCGGGTCCGGTGGTGGACGCCATTGAAGCCCGCTTTAAAGCC
CTCGGCGCGCCCGTGGAATTAATCAAAGTGCACAACACGCCGGACGGCAATTTC
CCCAACGGTATTCCTAACCCACTACTGCCGGAATGCCGCGACGACACCCGCAATG
CGGTCATCAAACACGGCGCGGATATGGGCATTGCTTTTGATGGCGATTTTGACCG
CTGTTTCCTGTTTGACGAAAAAGGGCAGTTTATTGAGGGCTACTACATTGTCGGC
CTGTTGGCAGAAGCATTCCTCGAAAAAAATCCCGGCGCGAAGATCATCCACGAT
CCACGTCTCTCCTGGAACACCGTTGATGTGGTGACTGCCGCAGGTGGCACGCCGG
TAATGTCGAAAACCGGACACGCCTTTATTAAAGAACGTATGCGCAAGGAAGACG
CCATCTATGGTGGCGAAATGAGCGCCCACCATTACTTCCGTGATTTCGCTTACTG
CGACAGCGGCATGATCCCGTGGCTGCTGGTCGCCGAACTGGTGTGCCTGAAAGA
TAAAACGCTGGGCGAACTGGTACGCGACCGGATGGCGGCGTTTCCGGCAAGCGG
TGAGATCAACAGCAAACTGGCGCAACCCGTTGAGGCGATTAACCGCGTGGAACA
GCATTTTAGCCGTGAGGCGCTGGCGGTGGATCGCACCGATGGCATCAGCATGAC
CTTTGCCGACTGGCGCTTTAACCTGCGCACCTCCAATACCGAACCGGTGGTGCGC
CTGAATGTGGAATCGCGCGGTGATGTGCCGCTGATGGAAGCGCGAACGCGAACT
CTGCTGACGTTGCTGAACGAGTAATGTCGGATCTTCCCTTACCCCACTGCGGGTA
AGGGGCTAATAACAGGAACAACGATGATTCCGGGGATCCGTCGACCTGCAGTTC
GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGAAGCAGCTCCAGCCTACAGT
TAACAAAGCGGCATATTGATATGAGCTTACGTGAAAAAACCATCAGCGGCGCGA
AGTGGTCGGCGATTGCCACGGTGATCATCATCGGCTTATTTTTGACACCAGACCA
ACTGGTAATTTATTTTTGACACCAGACCAACTGGTAATTTATTTTTGACACCAGAC
CAACTGGTTATTTTTGACACCAGACCAACTGGTTATTTTTGACACCAGACCAACT
GGCTCGGGCTGGTGCAGATGACCGTGCTGGCGCGGATTATCGACAACCACCAGT
TCGGCCTGCTTACCGTGTCGCTGGTGATTATCGCGCTGGCAGATACGCTTTCTGA
CTTCGGTATCGCTAACTCGATTATTCAGCGAAAAGAAATCAGTCACCTTGAACTC
ACCACGTTGTACTGGCTGAACGTCGGGCTGGGGATCGTGGTGTGCGTGGCGGTGT
TTTTGTTGAGTGATCTCATCGGCGACGTGCTGAATAACCCGGACCTGGCACCGTT
GATTAAAACATTATCGCTGGCGTTTGTGGTAATCCCCCACGGGCAACAGTTCCGC
GCGTTGATGCAAAAAGAGCTGGAGTTCAACAAAATCGGCATGATCGAAACCAGC
GCGGTGCTGGCGGGCTTCACTTGTACGGTGGTTAGCGCCCATTTCTGGCCGCTGG
CGATGACCGCGATCCTCGGTTATCTGGTCAATAGTGCGGTGAGAACGCTGCTGTT
TGGCTACTTTGGCCGCAAAATTTATCGCCCCGGTCTGCATTTCTCGCTGGCGTCGG
TGGCACCGAACTTACGCTTTGGTGCCTGGCTGACGGCGGACAGCATCATCAACTA
TCTCAATACCAACCTTTCAACGCTCGTGCTGGCGCGTATTCTCGGCGCGGGCGTG
GCAGGGGGATACAACCTGGCGTACAACGTGGCCGTTGTGCCACCGATGAAGCTG
AACCCAATCATCACCCGCGTGTTGTTTCCGGCATTCGCCAAAATTCAGGACGATA
CCGAAAAGCTGCGTGTTAACTTCTACAAGCTGCTGTCGGTAGTGGGGATTATCAA
CTTTCCGGCGCTGCTCGGGCTAATGGTGGTGTCGAATAACTTTGTACCGCTGGTC
TTTGGTGAGAAGTGGAACAGCATTATTCCGGTGCTGCAATTGCTGTGTGTGGTGG
GTCTGCTGCGCTCCG

[0076] Third, the magnitude of the cytoplasmic GDP-fucose pool was enhanced by the introduction of a null mutation into the *lon* gene. Lon is an ATP-dependant intracellular protease that is responsible for degrading RcsA, which is a positive transcriptional regulator of colanic acid biosynthesis in *E. coli* (Gottesman, S. & Stout, V. Mol Microbiol 5, 1599-1606 (1991)). In a *lon* null background, RcsA is stabilized, RcsA levels increase, the genes responsible for GDP-fucose synthesis in *E. coli* are up-regulated, and intracellular GDP-fucose concentrations are enhanced. The lon gene was almost entirely deleted and replaced by an inserted functional, wild-type, but promoter-less *E.coli lacZ*$^+$ gene (Δ*lon::(kan, lacZ*$^+$). λ Red recombineering was used to perform the construction. A schematic of the *kan*, *lacZ*$^+$ insertion into the *lon* locus is shown in Figure 8.

[0077] Genomic DNA sequence surrounding the lacZ+ insertion into the *lon* region in the *E.coli* strain is set forth below (SEQ ID NO: 15):

GTGGATGGAAGAGGTGGAAAAAGTGGTTATGGAGGAGTGGGTAATTGATGGTGA
AAGGAAAGGGTTGGTGATTTATGGGAAGGGGGAAGGGGAAGAGGGATGTGGTG
AATAATTAAGGATTGGGATAGAATTAGTTAAGGAAAAAGGGGGGATTTTATGTG
GGGTTTAATTTTTGGTGTATTGTGGGGGTTGAATGTGGGGGAAAGATGGGGATAT
AGTGAGGTAGATGTTAATAGATGGGGTGAAGGAGAGTGGTGTGATGTGATTAGG
TGGGGGAAATTAAAGTAAGAGAGAGGTGTATGATTGGGGGGATGGGTGGAGGT
GGAGTTGGAAGTTGGTATTGTGTAGAAAGTATAGGAAGTTGAGAGGGGTTTTGA
AGGTGAGGGTGGGGGAAGGAGTGAGGGGGGAAGGGGTGGTAAAGGAAGGGGA
AGAGGTAGAAAGGGAGTGGGGAGAAAGGGTGGTGAGGGGGGATGAATGTGA
GGTAGTGGGGTATGTGGAGAAGGGAAAAGGGAAGGGGAAAGAGAAAGGAGGTA
GGTTGGAGTGGGGTTAGATGGGGATAGGTAGAGTGGGGGGGTTTTATGGAGAGGA
AGGGAAGGGGAATTGGGAGGTGGGGGGGGGTGTGGTAAGGTTGGGAAGGGGTG
GAAAGTAAAGTGGATGGGTTTGTTGGGGGGAAGGATGTGATGGGGGAGGGGAT
GAAGATGTGATGAAGAGAGAGGATGAGGATGGTTTGGGATGATTGAAGAAGAT
GGATTGGAGGGAGGTTGTGGGGGGGGGTTGGGTGGAGAGGGTATTGGGGTATGAG
TGGGGAGAAGAGAGAATGGGGTGGTGTGATGGGGGGGGTGTTGGGGGGTGTGAGG
GGAGGGGGGGGGGGGTTGTTTTTGTGAAGAGGGAGGTGTGGGGTGGGGTGAATGA
AGTGGAGGAGGAGGGAGGGGGGGGTATGGTGGGTGGGGAGGAGGGGGGTTGGTT
GGGGAGGTGTGGTGGAGGTTGTGAGTGAAGGGGGAAGGGAGTGGGTGGTATTG
GGGGAAGTGGGGGGGGGAGGATGTGGTGTGATGTGAGGTTGGTGGTGGGGAGAA
AGTATGGATGATGGGTGATGGAATGGGGGGGGGTGGATAGGGTTGATGGGGGTAG
GTGGGGATTGGAGGAGGAAGGGAAAGATGGGATGGAGGGAGGAGGTAGTGGGA
TGGAAGGGGGTGTTGTGGATGAGGATGATGTGGAGGAAGAGGATGAGGGGGTG
GGGGGAGGGGAAGTGTTGGGGAGGGTGAAGGGGGGGATGGGGGAGGGGGAGGAT
GTGGTGGTGAGGGATGGGGATGGGTGGTTGGGGAATATGATGGTGGAAAATGGG
GGGTTTTGTGGATTGATGGAGTGTGGGGGGGGTGGGTGTGGGGGAGGGGTATGAG
GAGATAGGGTTGGGTAGGGGTGATATTGGTGAAGAGGTTGGGGGGGGAATGGG
GTGAGGGGTTGGTGGTGGTTTAGGGTATGGGGGGTGGGGATTGGGAGGGGATGG
GGTTGTATGGGGTTGTTGAGGAGTTGTTGTAATAAGGGGATGTTGAAGTTGGTAT
TGGGAAGTTGGTATTGTGTAGAAAGTATAGGAAGTTGGAAGGAGGTGGAGGGTA
GATAAAGGGGGGGGTTATTTTTGAGAGGAGAGGAAGTGGTAATGGTAGGGAGG
GGGGGTGAGGTGGAATTGGGGGGATAGTGAGGGGGTGGAGGAGTGGTGGGGAG
GAATGGGGATATGGAAAGGGTGGATATTGAGGGATGTGGGTTGTTGGGGGTGGA
GGAGATGGGGATGGGTGGTTTGGATGAGTTGGTGTTGAGTGTAGGGGGTGATGT
TGAAGTGGAAGTGGGGGGGGGAGTGGTGTGGGGGATAATTGAATTGGGGGGTG
GGGGAGGGGAGAGGGTTTTGGGTGGGGAAGAGGTAGGGGGTATAGATGTTGAG

AATGGGAGATGGGAGGGGTGAAAAGAGGGGGGAGTAAGGGGGTGGGGATAGTT
TTGTTGGGGGGGTAATGGGAGGGAGTTTAGGGGGTGTGGTAGGTGGGGGAGGTG
GGAGTTGAGGGGAATGGGGGGGGGATGGGGTGTATGGGTGGGGAGTTGAAGAT
GAAGGGTAATGGGGATTTGAGGAGTAGGATGAATGGGGTAGGTTTTGGGGGTGA
TAAATAAGGTTTTGGGGTGATGGTGGGAGGGGTGAGGGGTGGTAATGAGGAGGG
GATGAGGAAGTGTATGTGGGGTGGAGTGGAAGAAGGGTGGTTGGGGGTGGTAAT
GGGGGGGGGGGTTGGAGGGTTGGAGGGAGGGGTTAGGGTGAATGGGGGTGGGT
TGAGTTAGGGGAATGTGGTTATGGAGGGGTGGAGGGGTGAAGTGATGGGGGAG
GGGGGTGAGGAGTTGTTTTTTATGGGGAATGGAGATGTGTGAAAGAAAGGGTGA
GTGGGGGTTAAATTGGGAAGGGTTATTAGGGAGGTGGATGGAAAAATGGATTTG
GGTGGTGGTGAGATGGGGGATGGGGTGGGAGGGGGGGGGGAGGGTGAGAGTGA
GGTTTTGGGGGAGAGGGGAGTGGTGGGAGGGGGTGATGTGGGGGGGTTGTGAG
GATGGGGTGGGGTTGGGTTGGAGTAGGGGTAGTGTGAGGGAGAGTTGGGGGGG
GGTGTGGGGGTGGGGTAGTTGAGGGAGTTGAATGAAGTGTTTAGGTTGTGGAGG
GAGATGGAGAGGGAGTTGAGGGGTTGGGAGGGGGTTAGGATGGAGGGGGAGGA
TGGAGTGGAGGAGGTGGTTATGGGTATGAGGGAAGAGGTATTGGGTGGTGAGTT
GGATGGTTTGGGGGGATAAAGGGAAGTGGAAAAAGTGGTGGTGGTGTTTTGGTT
GGGTGAGGGGTGGATGGGGGGTGGGGTGGGGAAAGAGGAGAGGGTTGATAGAG
AAGTGGGGATGGTTGGGGGTATGGGGAAAATGAGGGGGGTAAGGGGAGGAGGG
GTTGGGGTTTTGATGATATTTAATGAGGGAGTGATGGAGGGAGTGGGAGAGGAA
GGGGGGGTGTAAAGGGGGATAGTGAGGAAAGGGGTGGGAGTATTTAGGGAAAG
GGGGAAGAGTGTTAGGGATGGGGTGGGGGTATTGGGAAAGGATGAGGGGGGGG
GTGTGTGGAGGTAGGGAAAGGGATTTTTTGATGGAGGATTTGGGGAGAGGGGGG
AAGGGGTGGTGTTGATGGAGGGGGGGGTAGATGGGGGAAATAATATGGGTGGG
GGTGGTGTGGGGTGGGGGGGGGTTGATAGTGGAGGGGGGGGGGAAGGATGGAGAG
ATTTGATGGAGGGATAGAGGGGGTGGTGATTAGGGGGGTGGGGTGATTGATTGG
GGAGGGAGGAGATGATGAGAGTGGGGTGATTAGGATGGGGGTGGAGGATTGGG
GTTAGGGGTTGGGTGATGGGGGGGTAGGGAGGGGGGATGATGGGTGAGAGGATT
GATTGGGAGGATGGGGTGGGTTTGAATATTGGGTTGATGGAGGAGATAGAGGGG
GTAGGGGTGGGAGAGGGTGTAGGAGAGGGGATGGTTGGGATAATGGGAAGAGG
GGAGGGGGTTAAAGTTGTTGTGGTTGATGAGGAGGATATGGTGGAGGATGGTGT
GGTGATGGATGAGGTGAGGATGGAGAGGATGATGGTGGTGAGGGTTAAGGGGT
GGAATGAGGAAGGGGTTGGGGTTGAGGAGGAGGAGAGGATTTTGAATGGGGAG
GTGGGGGAAAGGGAGATGGGAGGGTTGTGGTTGAATGAGGGTGGGGTGGGGGG
TGTGGAGTTGAAGGAGGGGAGGATAGAGATTGGGGATTTGGGGGGTGGAGAGTT
TGGGGTTTTGGAGGTTGAGAGGTAGTGTGAGGGGATGGGGATAAGGAGGAGGGT
GATGGATAATTTGAGGGGGGAAAGGGGGGGGTGGGGGTGGGGAGGTGGGTTTGA
GGGTGGGATAAAGAAAGTGTTAGGGGTAGGTAGTGAGGGAAGTGGGGGGAGAT
GTGAAGTTGAGGGTGGAGTAGAGGGGGGGTGAAATGATGATTAAAGGGAGTGG
GAAGATGGAAATGGGTGATTTGTGTAGTGGGTTTATGGAGGAAGGAGAGGTGAG
GGAAAATGGGGGTGATGGGGGAGATATGGTGATGTTGGAGATAAGTGGGGTGA
GTGGAGGGGAGGAGGATGAGGGGGAGGGGGTTTTGTGGGGGGGGTAAAAATGG
GGTGAGGTGAAATTGAGAGGGGAAAGGAGTGTGGTGGGGGTAAGGGAGGGAGG
GGGGGTTGGAGGAGAGATGAAAGGGGGAGTTAAGGGGATGAAAAATAATTGGG
GTGTGGGGTTGGTGTAGGGAGGTTTGATGAAGATTAAATGTGAGGGAGTAAGAA
GGGGTGGGATTGTGGGTGGGAAGAAAGGGGGGATTGAGGGTAATGGGATAGGT
GAGGTTGGTGTAGATGGGGGGATGGTAAGGGTGGATGTGGGAGTTTGAGGGGAG
GAGGAGAGTATGGGGGTGAGGAAGATGGGAGGGAGGGAGGTTTGGGGGAGGGG
TTGTGGTGGGGGAAAGGAGGGAAAGGGGGATTGGGGATTGAGGGTGGGGAAGT
GTTGGGAAGGGGGATGGGTGGGGGGGTGTTGGGTATTAGGGGAGGTGGGGAAA
GGGGGATGTGGTGGAAGGGGATTAAGTTGGGTAAGGGGAGGGTTTTGGGAGTGA

GGAGGTTGTAAAAGGAGGGGGAGTGAATGGGTAATGATGGTGATAGTAGGTTTG
GTGAGGTTGTGAGTGGAAAATAGTGAGGTGGGGGAAAATGGAGTAATAAAAAG
AGGGGTGGGAGGGTAATTGGGGGTTGGGAGGGTTTTTTTGTGTGGGTAAGTTAG
ATGGGGGATGGGGGTTGGGGTTATTAAGGGGTGTTGTAAGGGGATGGGTGGGGT
GATATAAGTGGTGGGGGTTGGTAGGTTGAAGGATTGAAGTGGGATATAAATTAT
AAAGAGGAAGAGAAGAGTGAATAAATGTGAATTGATGGAGAAGATTGGTGGAG
GGGGTGATATGTGTAAAGGTGGGGGTGGGGGTGGGTTAGATGGTATTATTGGTT
GGGTAAGTGAATGTGTGAAAGAAGG

[0078] Preferably, the genomic DNA sequence surrounding the lacZ+ insertion into the *lon* region in the *E.coli* strain is as set forth below (SEQ ID NO: 19) (the ribosome binding site is designated by lowercase, the codon complementary to the 5' ATG start codon *lacZ*, and the codon complementary to the STOP codon is in bold and underlined):

GTCCATGGAAGACGTCGAAAAAGTGGTTATCGACGAGTCGGTAATTGATGGTCA
AAGCAAACCGTTGCTGATTTATGGCAAGCCGGAAGCGCAACAGGCATCTGGTGA
ATAATTAACCATTCCCATACAATTAGTTAACCAAAAAGGGGGGATTTTATCTCCC
CTTTAATTTTTCCTCTATTCTCGGCGTTGAATGTGGGGGAAACATCCCCATATACT
GACGTACATGTTAATAGATGGCGTGAAGCACAGTCGTGTCATCTGATTACCTGGC
GGAAATTAAACTAAGAGAGAGCTCTATGATTCCGGGGATCCGTCGACCTGCAGT
TCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCAGAGCGCTTTTGAAGCTCA
CGCTGCCGCAAGCACTCAGGGCGCAAGGGCTGCTAAAGGAAGCGGAACACGTAG
AAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAGCTACTGGGCT
ATCTGGACAAGGGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGG
GCTTACATGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGA
ATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTG
GATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAA
GAGACAGGATGAGGATCGTTTCGCATGATTGAACAAGATGGATTGCACGCAGGT
TCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACA
ATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTC
TTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGC
GCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTT
GTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGAT
CTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAA
TGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAA
ACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGA
TGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCT
CAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTG
CTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGC
CGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTG
CTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGC
CGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTAAT
AAGGGGATCTTGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAA
CTTCGAAGCAGCTCCAGCCTACATAAGCGGCCGC**TTA**TTTTTGACACCAGACCAA
CTGGTAATGGTAGCGACCGGCGCTCAGCTGGAATTCCGCCGATACTGACGGGCTC
CAGGAGTCGTCGCCACCAATCCCCATATGGAAACCGTCGATATTCAGCCATGTGC
CTTCTTCCGCGTGCAGCAGATGGCGATGGCTGGTTTCCATCAGTTGCTGTTGACT
GTAGCGGCTGATGTTGAACTGGAAGTCGCCGCGCCACTGGTGTGGGCCATAATTC
AATTCGCGCGTCCCGCAGCGCAGACCGTTTTCGCTCGGGAAGACGTACGGGGTAT

ACATGTCTGACAATGGCAGATCCCAGCGGTCAAAACAGGCGGCAGTAAGGCGGT
CGGGATAGTTTTCTTGCGGCCCTAATCCGAGCCAGTTTACCCGCTCTGCTACCTGC
GCCAGCTGGCAGTTCAGGCCAATCCGCGCCGGATGCGGTGTATCGCTCGCCACTT
CAACATCAACGGTAATCGCCATTTGACCACTACCATCAATCCGGTAGGTTTTCCG
GCTGATAAATAAGGTTTTCCCCTGATGCTGCCACGCGTGAGCGGTCGTAATCAGC
ACCGCATCAGCAAGTGTATCTGCCGTGCACTGCAACAACGCTGCTTCGGCCTGGT
AATGGCCCGCCGCCTTCCAGCGTTCGACCCAGGCGTTAGGGTCAATGCGGGTCGC
TTCACTTACGCCAATGTCGTTATCCAGCGGTGCACGGGTGAACTGATCGCGCAGC
GGCGTCAGCAGTTGTTTTTTATCGCCAATCCACATCTGTGAAAGAAAGCCTGACT
GGCGGTTAAATTGCCAACGCTTATTACCCAGCTCGATGCAAAAATCCATTTCGCT
GGTGGTCAGATGCGGGATGGCGTGGGACGCGGCGGGGAGCGTCACACTGAGGTT
TTCCGCCAGACGCCACTGCTGCCAGGCGCTGATGTGCCCGGCTTCTGACCATGCG
GTCGCGTTCGGTTGCACTACGCGTACTGTGAGCCAGAGTTGCCCGGCGCTCTCCG
GCTGCGGTAGTTCAGGCAGTTCAATCAACTGTTTACCTTGTGGAGCGACATCCAG
AGGCACTTCACCGCTTGCCAGCGGCTTACCATCCAGCGCCACCATCCAGTGCAGG
AGCTCGTTATCGCTATGACGGAACAGGTATTCGCTGGTCACTTCGATGGTTTGCC
CGGATAAACGGAACTGGAAAAACTGCTGCTGGTGTTTTGCTTCCGTCAGCGCTGG
ATGCGGCGTGCGGTCGGCAAAGACCAGACCGTTCATACAGAACTGGCGATCGTT
CGGCGTATCGCCAAAATCACCGCCGTAAGCCGACCACGGGTTGCCGTTTTCATCA
TATTTAATCAGCGACTGATCCACCCAGTCCCAGACGAAGCCGCCCTGTAAACGGG
GATACTGACGAAACGCCTGCCAGTATTTAGCGAAACCGCCAAGACTGTTACCCAT
CGCGTGGGCGTATTCGCAAAGGATCAGCGGGCGCGTCTCTCCAGGTAGCGAAAG
CCATTTTTTGATGGACCATTTCGGCACAGCCGGGAAGGGCTGGTCTTCATCCACG
CGCGCGTACATCGGGCAAATAATATCGGTGGCCGTGGTGTCGGCTCCGCCGCCTT
CATACTGCACCGGGCGGGAAGGATCGACAGATTTGATCCAGCGATACAGCGCGT
CGTGATTAGCGCCGTGGCCTGATTCATTCCCCAGCGACCAGATGATCACACTCGG
GTGATTACGATCGCGCTGCACCATTCGCGTTACGCGTTCGCTCATCGCCGGTAGC
CAGCGCGGATCATCGGTCAGACGATTCATTGGCACCATGCCGTGGGTTTCAATAT
TGGCTTCATCCACCACATACAGGCCGTAGCGGTCGCACAGCGTGTACCACAGCG
GATGGTTCGGATAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAG
CAGGATATCCTGCACCATCGTCTGCTCATCCATGACCTGACCATGCAGAGGATGA
TGCTCGTGACGGTTAACGCCTCGAATCAGCAACGGCTTGCCGTTCAGCAGCAGCA
GACCATTTTCAATCCGCACCTCGCGGAAACCGACATCGCAGGCTTCTGCTTCAAT
CAGCGTGCCGTCGGCGGTGTGCAGTTCAACCACCGCACGATAGAGATTCGGGAT
TTCGGCGCTCCACAGTTTCGGGTTTTCGACGTTCAGACGTAGTGTGACGCGATCG
GCATAACCACCACGCTCATCGATAATTTCACCGCCGAAAGGCGCGGTGCCGCTG
GCGACCTGCGTTTCACCCTGCCATAAAGAAACTGTTACCCGTAGGTAGTCACGCA
ACTCGCCGCACATCTGAACTTCAGCCTCCAGTACAGCGCGGCTGAAATCATCATT
AAAGCGAGTGGCAACATGGAAATCGCTGATTTGTGTAGTCGGTTTATGCAGCAA
CGAGACGTCACGGAAAATGCCGCTCATCCGCCACATATCCTGATCTTCCAGATAA
CTGCCGTCACTCCAGCGCAGCACCATCACCGCGAGGCGGTTTTCTCCGGCGCGTA
AAAATGCGCTCAGGTCAAATTCAGACGGCAAACGACTGTCCTGGCCGTAACCGA
CCCAGCGCCCGTTGCACCACAGATGAAACGCCGAGTTAACGCCATCAAAAATAA
TTCGCGTCTGGCCTTCCTGTAGCCAGCTTTCATCAACATTAAATGTGAGCGAGTA
ACAACCCGTCGGATTCTCCGTGGGAACAAACGGCGGATTGACCGTAATGGGATA
GGTCACGTTGGTGTAGATGGGCGCATCGTAACCGTGCATCTGCCAGTTTGAGGGG
ACGACGACAGTATCGGCCTCAGGAAGATCGCACTCCAGCCAGCTTTCCGGCACC
GCTTCTGGTGCCGGAAACCAGGCAAAGCGCCATTCGCCATTCAGGCTGCGCAACT
GTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAG
GGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCAC
GACGTTGTAAAACGACGGCCAGTGAATCCGTAATCATGGTCATagtaggtttcctCAGGT

TGTGACTGCAAAATAGTGACCTCGCGCAAAATGCACTAATAAAAACAGGGCTGG
CAGGCTAATTCGGGCTTGCCAGCCTTTTTTTGTCTCGCTAAGTTAGATGGCGGATC
GGGCTTGCCCTTATTAAGGGGTGTTGTAAGGGGATGGCTGGCCTGATATAACTGC
TGCGCGTTCGTACCTTGAAGGATTCAAGTGCGATATAAATTATAAAGAGGAAGA
GAAGAGTGAATAAATCTCAATTGATCGACAAGATTGCTGCAGGGGCTGATATCT
CTAAAGCTGCGGCTGGCCGTGCGTTAGATGCTATTATTGCTTCCGTAACTGAATC
TCTGAAAGAAGG

[0079] The nucleic acid sequence that encodes the lacZ protein is set forth below (SEQ ID NO: 20) (the ATG start codon is underlined and the STOP codon is in bold and underlined):

ATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAA
ACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTG
GCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCT
GAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAG
CTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGG
CAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCCATTACGG
TCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTT
AATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGC
GTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGG
ACAGTCGTTTGCCGTCTGAATTTGACCTGAGCGCATTTTTACGCGCCGGAGAAAA
CCGCCTCGCGGTGATGGTGCTGCGCTGGAGTGACGGCAGTTATCTGGAAGATCA
GGATATGTGGCGGATGAGCGGCATTTTCCGTGACGTCTCGTTGCTGCATAAACCG
ACTACACAAATCAGCGATTTCCATGTTGCCACTCGCTTTAATGATGATTTCAGCC
GCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACG
GGTAACAGTTTCTTTATGGCAGGGTGAAACGCAGGTCGCCAGCGGCACCGCGCC
TTTCGGCGGTGAAATTATCGATGAGCGTGGTGGTTATGCCGATCGCGTCACACTA
CGTCTGAACGTCGAAAACCCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTAT
CGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCC
TGCGATGTCGGTTTCCGCGAGGTGCGGATTGAAAATGGTCTGCTGCTGCTGAACG
GCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGAGCATCATCCTCTGCATGG
TCAGGTCATGGATGAGCAGACGATGGTGCAGGATATCCTGCTGATGAAGCAGAA
CAACTTTAACGCCGTGCGCTGTTCGCATTATCCGAACCATCCGCTGTGGTACACG
CTGTGCGACCGCTACGGCCTGTATGTGGTGGATGAAGCCAATATTGAAACCCACG
GCATGGTGCCAATGAATCGTCTGACCGATGATCCGCGCTGGCTACCGGCGATGA
GCGAACGCGTAACGCGAATGGTGCAGCGCGATCGTAATCACCCGAGTGTGATCA
TCTGGTCGCTGGGGAATGAATCAGGCCACGGCGCTAATCACGACGCGCTGTATC
GCTGGATCAAATCTGTCGATCCTTCCCGCCCGGTGCAGTATGAAGGCGGCGGAGC
CGACACCACGGCCACCGATATTATTTGCCCGATGTACGCGCGCGTGGATGAAGA
CCAGCCCTTCCCGGCTGTGCCGAAATGGTCCATCAAAAAATGGCTTTCGCTACCT
GGAGAGACGCGCCCGCTGATCCTTTGCGAATACGCCCACGCGATGGGTAACAGT
CTTGGCGGTTTCGCTAAATACTGGCAGGCGTTTCGTCAGTATCCCCGTTTACAGG
GCGGCTTCGTCTGGGACTGGGTGGATCAGTCGCTGATTAAATATGATGAAAACG
GCAACCCGTGGTCGGCTTACGGCGGTGATTTTGGCGATACGCCGAACGATCGCCA
GTTCTGTATGAACGGTCTGGTCTTTGCCGACCGCACGCCGCATCCAGCGCTGACG
GAAGCAAAACACCAGCAGCAGTTTTTCCAGTTCCGTTTATCCGGGCAAACCATCG
AAGTGACCAGCGAATACCTGTTCCGTCATAGCGATAACGAGCTCCTGCACTGGAT
GGTGGCGCTGGATGGTAAGCCGCTGGCAAGCGGTGAAGTGCCTCTGGATGTCGC
TCCACAAGGTAAACAGTTGATTGAACTGCCTGAACTACCGCAGCCGGAGAGCGC

CGGGCAACTCTGGCTCACAGTACGCGTAGTGCAACCGAACGCGACCGCATGGTC
AGAAGCCGGGCACATCAGCGCCTGGCAGCAGTGGCGTCTGGCGGAAAACCTCAG
TGTGACGCTCCCCGCCGCGTCCCACGCCATCCCGCATCTGACCACCAGCGAAATG
GATTTTTGCATCGAGCTGGGTAATAAGCGTTGGCAATTTAACCGCCAGTCAGGCT
TTCTTTCACAGATGTGGATTGGCGATAAAAAACAACTGCTGACGCCGCTGCGCGA
TCAGTTCACCCGTGCACCGCTGGATAACGACATTGGCGTAAGTGAAGCGACCCG
CATTGACCCTAACGCCTGGGTCGAACGCTGGAAGGCGGCGGGCCATTACCAGGC
CGAAGCAGCGTTGTTGCAGTGCACGGCAGATACACTTGCTGATGCGGTGCTGATT
ACGACCGCTCACGCGTGGCAGCATCAGGGGAAAACCTTATTTATCAGCCGGAAA
ACCTACCGGATTGATGGTAGTGGTCAAATGGCGATTACCGTTGATGTTGAAGTGG
CGAGCGATACACCGCATCCGGCGCGGATTGGCCTGAACTGCCAGCTGGCGCAGG
TAGCAGAGCGGGTAAACTGGCTCGGATTAGGGCCGCAAGAAAACTATCCCGACC
GCCTTACTGCCGCCTGTTTTGACCGCTGGGATCTGCCATTGTCAGACATGTATACC
CCGTACGTCTTCCCGAGCGAAAACGGTCTGCGCTGCGGGACGCGCGAATTGAATT
ATGGCCCACACCAGTGGCGCGGCGACTTCCAGTTCAACATCAGCCGCTACAGTCA
ACAGCAACTGATGGAAACCAGCCATCGCCATCTGCTGCACGCGGAAGAAGGCAC
ATGGCTGAATATCGACGGTTTCCATATGGGGATTGGTGGCGACGACTCCTGGAGC
CCGTCAGTATCGGCGGAATTCCAGCTGAGCGCCGGTCGCTACCATTACCAGTTGG
TCTGGTGTCAAAAA**TAA**

[0080] Fourth, a *thyA* (thymidylate synthase) mutation was introduced into the strain by P1 transduction. In the absence of exogenous thymidine, *thyA.* strains are unable to make DNA and die. The defect can be complemented in *trans* by supplying a wild-type *thyA* gene on a multicopy plasmid (Belfort, M., Maley, G.F., and Maley, F. (1983). Proc Natl Acad Sci U S A 80, 1858-861). This complementation was used here as a means of plasmid maintenance.

[0081] An additional modification that is useful for increasing the cytoplasmic pool of free lactose (and hence the final yield of 2'-FL) is the incorporation of a *lacA* mutation. LacA is a lactose acetyltransferase that is only active when high levels of lactose accumulate in the *E. coli* cytoplasm. High intracellular osmolarity (*e.g.*, caused by a high intracellular lactose pool) can inhibit bacterial growth, and *E. coli* has evolved a mechanism for protecting itself from high intra cellular osmlarity caused by lactose by "tagging" excess intracellular lactose with an acetyl group using LacA, and then actively expelling the acetyl-lactose from the cell (Danchin, A. Bioessays 31, 769-773 (2009)). Production of acetyl-lactose in *E. coli* engineered to produce 2'-FL or other human milk oligosaccharides is therefore undesirable: it reduces overall yield. Moreover, acetyl-lactose is a side product that complicates oligosaccharide purification schemes. The incorporation of a *lacA* mutation resolves these problems. Sub-optimal production of fucosylated oligosaccharides occurs in strains lacking either or both of the mutations in the colanic acid pathway and the Ion protease. Diversion of lactose into a side product (acetyl-lactose) occurs in strains that don't contain the lacA mutation. A schematic of the *lacA* deletion and corresponding genomic sequence is provided above (SEQ ID NO: 13).

[0082] The strain used to test the different α(1,2) FT candidates incorporates all the above genetic modifications and has the following genotype: Δ*ampC*::$P_{trp}{}^{B}$*cI*, Δ(*lacI-lacZ*)::*FRT*, P_{laclq}*lacY*$^{+}$, Δ*wcaJ*::FRT, *thyA*::*Tn*10, *Δlon*:(*npt3*, *lacZ*$^{+}$), *ΔlacA*

[0083] The *E. coli* strains harboring the different α(1,2) FT candidate expression plasmids were analyzed. Strains were grown in selective media (lacking thymidine) to early exponential phase. Lactose was then added to a final concentration of 1%, and tryptophan (200 μM) was added to induce expression of each candidate α(1,2) FT from the P_L promoter. At the end of the induction period (-20 h) equivalent OD 600 units of each strain were harvested. Lysates were prepared and analyzed for the presence of 2'-FL by thin layer chromatography (TLC). As shown in Figure 3A, a control strain producing FutC-Myc was efficient in the biosynthesis of 2'-FL and also produced a smaller amount of the tetrasaccharide lactodifucotetraose (LDFT). The previously characterized α(1,2) FT WbsJ from *E. coli* 0128:B12 was also capable of catalyzing 2'-FL synthesis, although only at ~30% the level produced by FutC-Myc (Figure 3A, lanes 5 and 6). Wb1A (derived from *V. cholerae* 022) was able to promote 2'-FL synthesis, although at a significantly lower level compared to FutC (Figure 3A, lanes 7 and 8). This result was not unexpected, as *V. cholerae* 022 does not incorporate fucose into cell surface glycans (Cox, A.D. et al. (1997). Carbohydr Res 304, 191-208). The strain producing WbgL (derived from *E. coli* strain 0126) from plasmid pG204 synthesized a significant amount of 2'-FL, approximately ~75% of the amount produced by FutC-Myc (Figure 3A, lanes 9 and 10). WbgL was also capable of synthesizing LDFT. The strain producing FutL (derived from *H. mustelae* ATCC 43772) from plasmid pG216 was capable of directing the synthesis of robust amounts of 2'-FL, comparable to the levels obtained utilizing FutC-Myc and WbgL (Figure 3B, lanes 7 and 8). Furthermore, a strain producing FutN (derived from *B. vulgatus* ATCC 8482) from plasmid pG217 also produced significant amounts

of 2'-FL, approximately ~50% the amount produced by FutC-Myc (Figure 3C, lanes 5 and 6). FutN is derived from the commensal bacterium *B. vulgatus*, and therefore may not be subject to the same concerns associated with utilization of an α(1,2) FT obtained from a pathogenic bacterium for the production of a food additive.

[0084] A map of plasmid pG204 is shown in Figure 11. The sequence of plasmid pG204 is set forth below (SEQ ID NO: 16) (the codon complementary to the ATG start codon for *E. coli 0126 WbgL* is underlined; and the codon complementary to the STOP codon is underlined and in bold):

AATTCTAAAAATTGATTGAATGTATGCAAATAAATGCATACACCATAGGTGTGGT
TTAATTTGATGCCCTTTTTCAGGGCTGGAATGTGTAAGAGCGGGGTTATTTATGCT
GTTGTTTTTTTGTTACTCGGGAAGGGCTTTACCTCTTCCGCATAAACGCTTCCATC
AGCGTTTATAGTTAAAAAAATCTTTCGGAACTGGTTTTGCGCTTACCCCAACCAA
CAGGGGATTTGCTGCTTTCCATTGAGCCTGTTTCTCTGCGCGACGTTCGCGGCGG
CGTGTTTGTGCATCCATCTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTGTGGCA
GTTGTAGTCCTGAACGAAAACCCCCCGCGATTGGCACATTGGCAGCTAATCCGGA
ATCGCACTTACGGCCAATGCTTCGTTTCGTATCACACACCCCAAAGCCTTCTGCTT
TGAATGCTGCCCTTCTTCAGGGCTTAATTTTTAAGAGCGTCACCTTCATGGTGGTC
AGTGCGTCCTGCTGATGTGCTCAGTATCACCGCCAGTGGTATTTATGTCAACACC
GCCAGAGATAATTTATCACCGCAGATGGTTATCTGTATGTTTTTTATATGAATTTA
TTTTTTGCAGGGGGGCATTGTTTGGTAGGTGAGAGATCAATTCTGCATTAATGAA
TCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTC
GCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCAC
TCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAAC
ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCT
GGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCA
AGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCT
GGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTC
CGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATC
TCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGT
TCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTA
AGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCG
AGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACA
CTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAA
AAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTT
TTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCT
TTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGA
TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAA
ATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAC
CAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCAT
AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCT
GGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTAT
CAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTT
TATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTC
GCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCA
CGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAG
TTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGAT
CGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTG
CATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTA
CTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCG
GCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATC
ATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGAT
CCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTC
ACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGG
AATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTAT
TGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTT
AGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTG
ACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCAC
GAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATG
CAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAA

GCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTAT
GCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGTGAAATAC
CGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCTCCTCAACCTGTATA
TTCGTAAACCACGCCCAATGGGAGCTGTCTCAGGTTTGTTCCTGATTGGTTACGG
CGCGTTTCGCATCATTGTTGAGTTTTTCCGCCAGCCCGACGCGCAGTTTACCGGTG
CCTGGGTGCAGTACATCAGCATGGGGCAAATTCTTTCCATCCCGATGATTGTCGC
GGGTGTGATCATGATGGTCTGGGCATATCGTCGCAGCCCACAGCAACACGTTTCC
TGAGGAACCATGAAACAGTATTTAGAACTGATGCAAAAAGTGCTCGACGAAGGC
ACACAGAAAAACGACCGTACCGGAACCGGAACGCTTTCCATTTTTGGTCATCAG
ATGCGTTTTAACCTGCAAGATGGATTCCCGCTGGTGACAACTAAACGTTGCCACC
TGCGTTCCATCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTAACATTGC
TTATCTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATGAAAACGG
CGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAACGCCAGATGG
TCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGAAAAACGACCCGGA
TTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGCGAACTGGATAAAATGGCG
CTGGCACCGTGCCATGCATTCTTCCAGTTCTATGTGGCAGACGGCAAACTCTCTT
GCCAGCTTTATCAGCGCTCCTGTGACGTCTTCCTCGGCCTGCCGTTCAACATTGCC
AGCTACGCGTTATTGGTGCATATGATGGCGCAGCAGTGCGATCTGGAAGTGGGT
GATTTTGTCTGGACCGGTGGCGACACGCATCTGTACAGCAACCATATGGATCAAA
CTCATCTGCAATTAAGCCGCGAACCGCGTCCGCTGCCGAAGTTGATTATCAAACG
TAAACCCGAATCCATCTTCGACTACCGTTTCGAAGACTTTGAGATTGAAGGCTAC
GATCCGCATCCGGGCATTAAAGCGCCGGTGGCTATCTAATTACGAAACATCCTGC
CAGAGCCGACGCCAGTGTGCGTCGGTTTTTTTACCCTCCGTTAAATTCTTCGAGA
CGCCTTCCCGAAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCG
ATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCA
AGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACG
ACGGCCAGTGCCAAGCTTTCTTTAATGAAGCAGGGCATCAGGACGGTATCTTTGT
GGAGAAAGCAGAGTAATCTTATTCAGCCTGACTGGTGGGAAACCACCAGTCAGA
ATGTGTTAGCGCATGTTGACAAAAATACCATTAGTCACATTATCCGTCAGTCGGA
CGACATGGTAGATAACCTGTTTATTATGCGTTTTGATCTTACGTTTAATATTACCT
TTATGCGATGAAACGGTCTTGGCTTTGATATTCATTTGGTCAGAGATTTGAATGG
TTCCCTGACCTGCCATCCACATTCGCAACATACTCGATTCGGTTCGGCTCAATGAT
AACGTCGGCATATTTAAAAACGAGGTTATCGTTGTCTCTTTTTTCAGAATATCGCC
AAGGATATCGTCGAGAGATTCCGGTTTAATCGATTTAGAACTGATCAATAAATTT
TTTCTGACCAATAGATATTCATCAAAATGAACATTGGCAATTGCCATAAAAACGA
TAAATAACGTATTGGGATGTTGATTAATGATGAGCTTGATACGCTGACTGTTAGA
AGCATCGTGGATGAAACAGTCCTCATTAATAAACACCACTGAAGGGCGCTGTGA
ATCACAAGCTATGGCAAGGTCATCAACGGTTTCAATGTCGTTGATTTCTCTTTTTT
TAACCCCTCTACTCAACAGATACCCGGTTAAACCTAGTCGGGTGTAACTACATAA
ATCCATAATAATCGTTGACATGGCATACCCTCACTCAATGCGTAACGATAATTCC
CCTTACCTGAATATTTCATCATGACTAAACGGAACAACATGGGTCACCTAATGCG
CCACTCTCGCGATTTTTCAGGCGGACTTACTATCCCGTAAAGTGTTGTATAATTTG
CCTGGAATTGTCTTAAAGTAAAGTAAATGTTGCGATATGTGAGTGAGCTTAAAAC
AAATATTTCGCTGCAGGAGTATCCTGGAAGATGTTCGTgAGAAGCTTACTGCTCA
CAAGAAAAAAGGCACGTCATCTGACGTGCCTTTTTTATTTGTACTACCCTGTACG
ATTACTGCAGCTCGAG**CTA**ACACGAGCTATGTTTATCCACGTTTATCCAGTGATT
GACTATGGGGATATAAGTATTTTTTGGAGTTATATCGTACCAAGGAGTAGGATAA
ATAACAATCTGTGACGCTGATGTACCTAAATAAGCCCCCCACCAACTAAAACTAC
TATTCGCTATAATATGATGGTTAGCTAAGCTCATTAACCATAAATCTTCTTCTTGT
GATAAATCTTCTGAATAATATATATTATATTTTTTACTGAGTAATGTTTCGATATT
TTCTTTACACCAAAAAATATCATCACTGAAAATAAACACGTCACGTATCATTGCC

AAATCGCGTATTTTATTTAAAGCTTTTTTGTAATACTCTAACGAACAAACGCCAT
GAGTTAAAGTAGCTGTTTTGTTTTTTATATAATCTCCTCTTCTTATATGAATAGAA
AGTGATGATTGAGATTCAAGAATTTTTGCTGCAAGTAAATTTGCTTGTTCAGACA
CATTCTTTGGAATAAAAAATTCTTTTAGATCTAATATATGTTTATGGAAAAAGTG
CTCAGATTGCCAATACCCTATATATTTTTTGGATTTCCATTTTTGCGCTATATATTC
AAAATCATAACCATAGGCATGAAATTCATTGCAAAAACCTAAAAAAAGAAAGAT
TTCAGGATATAATCTTGACCCACGAACCAAAAATTTATAAATATTATTAATTTTT
GGTGTGTAATACTGTAAATATTCCTCTGGAATTTGTAGATTGTTTAGCCTGTAACC
ACCATGATCATCATTTTCAGCATAATGACTTATATCAAAATATAATGGTGTCCCA
TTAATTTTGGAAAGCGCATACCCAAATGAGAACTGAAAAAGTTGATTTCCAAGTC
CGCCTTGTAATCTTATAATAGACATTATATCTCCTTCTTG

[0085] The nucleic acid sequence that encodes *E. coli O126* WbgL protein from plasmid G204 is set forth below (SEQ ID NO: 21) (the ATG start codon is underlined and the STOP codon is in bold and underlined):

ATGTCTATTATAAGATTACAAGGCGGACTTGGAAATCAACTTTTTCAGTTCTCATT
TGGGTATGCGCTTTCCAAAATTAATGGGACACCATTATATTTTGATATAAGTCAT
TATGCTGAAAATGATGATCATGGTGGTTACAGGCTAAACAATCTACAAATTCCAG
AGGAATATTTACAGTATTACACACCAAAAATTAATAATATTTATAAATTTTTGGT
TCGTGGGTCAAGATTATATCCTGAAATCTTTCTTTTTTTAGGTTTTTGCAATGAAT
TTCATGCCTATGGTTATGATTTTGAATATATAGCGCAAAAATGGAAATCCAAAAA
ATATATAGGGTATTGGCAATCTGAGCACTTTTTCCATAAACATATATTAGATCTA
AAAGAATTTTTTATTCCAAAGAATGTGTCTGAACAAGCAAATTTACTTGCAGCAA
AAATTCTTGAATCTCAATCATCACTTTCTATTCATATAAGAAGAGGAGATTATAT
AAAAAACAAAACAGCTACTTTAACTCATGGCGTTTGTTCGTTAGAGTATTACAAA
AAAGCTTTAAATAAAATACGCGATTTGGCAATGATACGTGACGTGTTTATTTTCA
GTGATGATATTTTTTGGTGTAAAGAAAATATCGAAACATTACTCAGTAAAAAATA
TAATATATATTATTCAGAAGATTTATCACAAGAAGAAGATTTATGGTTAATGAGC
TTAGCTAACCATCATATTATAGCGAATAGTAGTTTTAGTTGGTGGGGGGCTTATT
TAGGTACATCAGCGTCACAGATTGTTATTTATCCTACTCCTTGGTACGATATAACT
CCAAAAAATACTTATATCCCCATAGTCAATCACTGGATAAACGTGGATAAACATA
GCTCGTGT**TAG**

[0086] A map of plasmid pG216 is shown in Figure 9. The sequence of plasmid pG216 is set forth below (SEQ ID NO: 17) (the codon complementary to the 5' ATG start codon for *H. mustelae FutL* is underlined, and the codon complementary to the STOP codon is in bold and underlined):

TCTAGAATTCTAAAAATTGATTGAATGTATGCAAATAAATGCATACACCA
TAGGTGTGGTTTAATTTGATGCCCTTTTTCAGGGCTGGAATGTGTAAGAGCGGGG
TTATTTATGCTGTTGTTTTTTTGTTACTCGGGAAGGGCTTTACCTCTTCCGCATAA
ACGCTTCCATCAGCGTTTATAGTTAAAAAAATCTTTCGGAACTGGTTTTGCGCTTA
CCCCAACCAACAGGGGATTTGCTGCTTTCCATTGAGCCTGTTTCTCTGCGCGACG
TTCGCGGCGGCGTGTTTGTGCATCCATCTGGATTCTCCTGTCAGTTAGCTTTGGTG
GTGTGTGGCAGTTGTAGTCCTGAACGAAAACCCCCCGCGATTGGCACATTGGCAG
CTAATCCGGAATCGCACTTACGGCCAATGCTTCGTTTCGTATCACACACCCCAAA
GCCTTCTGCTTTGAATGCTGCCCTTCTTCAGGGCTTAATTTTTAAGAGCGTCACCT
TCATGGTGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCACCGCCAGTGGTATTT

ATGTCAACACCGCCAGAGATAATTTATCACCGCAGATGGTTATCTGTATGTTTTTT
ATATGAATTTATTTTTTGCAGGGGGGCATTGTTTGGTAGGTGAGAGATCAATTCT
GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTC
TTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCG
GTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAAC
GCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAA
GGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAA
AATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAG
GCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTAC
CGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAC
GCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCA
CGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAG
TCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGG
ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCT
AACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAG
TTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGG
TAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCT
CAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACT
CACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCT
TTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGG
TCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTAT
TTCGTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGA
GGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCG
GCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGT
GGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTA
GAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGG
CATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAAC
GATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTT
CGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTT
ATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGT
GACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAG
TTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTA
AAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTAC
CGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGC
ATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCC
GCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTT
TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATT
TGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAA
AGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAAT
AGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACC
TCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGG
GAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTG
GCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGT
GTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCTCCTCA
ACCTGTATATTCGTAAACCACGCCCAATGGGAGCTGTCTCAGGTTTGTTCCTGAT
TGGTTACGGCGCGTTTCGCATCATTGTTGAGTTTTTCCGCCAGCCCGACGCGCAG
TTTACCGGTGCCTGGGTGCAGTACATCAGCATGGGGCAAATTCTTTCCATCCCGA
TGATTGTCGCGGGTGTGATCATGATGGTCTGGGCATATCGTCGCAGCCCACAGCA
ACACGTTTCCTGAGGAACCATGAAACAGTATTTAGAACTGATGCAAAAAGTGCT
CGACGAAGGCACACAGAAAAACGACCGTACCGGAACCGGAACGCTTTCCATTTT
TGGTCATCAGATGCGTTTTAACCTGCAAGATGGATTCCCGCTGGTGACAACTAAA

CGTTGCCACCTGCGTTCCATCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACA
CTAACATTGCTTATCTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGA
TGAAAACGGCGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAAC
GCCAGATGGTCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGAAAAA
CGACCCGGATTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGCGAACTGGAT
AAAATGGCGCTGGCACCGTGCCATGCATTCTTCCAGTTCTATGTGGCAGACGGCA
AACTCTCTTGCCAGCTTTATCAGCGCTCCTGTGACGTCTTCCTCGGCCTGCCGTTC
AACATTGCCAGCTACGCGTTATTGGTGCATATGATGGCGCAGCAGTGCGATCTGG
AAGTGGGTGATTTTGTCTGGACCGGTGGCGACACGCATCTGTACAGCAACCATAT
GGATCAAACTCATCTGCAATTAAGCCGCGAACCGCGTCCGCTGCCGAAGTTGATT
ATCAAACGTAAACCCGAATCCATCTTCGACTACCGTTTCGAAGACTTTGAGATTG
AAGGCTACGATCCGCATCCGGGCATTAAAGCGCCGGTGGCTATCTAATTACGAA
ACATCCTGCCAGAGCCGACGCCAGTGTGCGTCGGTTTTTTTACCCTCCGTTAAATT
CTTCGAGACGCCTTCCCGAAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGG
GAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGA
TGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTT
GTAAAACGACGGCCAGTGCCAAGCTTTCTTTAATGAAGCAGGGCATCAGGACGG
TATCTTTGTGGAGAAAGCAGAGTAATCTTATTCAGCCTGACTGGTGGGAAACCAC
CAGTCAGAATGTGTTAGCGCATGTTGACAAAAATACCATTAGTCACATTATCCGT
CAGTCGGACGACATGGTAGATAACCTGTTTATTATGCGTTTTGATCTTACGTTTAA
TATTACCTTTATGCGATGAAACGGTCTTGGCTTTGATATTCATTTGGTCAGAGATT
TGAATGGTTCCCTGACCTGCCATCCACATTCGCAACATACTCGATTCGGTTCGGC
TCAATGATAACGTCGGCATATTTAAAAACGAGGTTATCGTTGTCTCTTTTTTCAGA
ATATCGCCAAGGATATCGTCGAGAGATTCCGGTTTAATCGATTTAGAACTGATCA
ATAAATTTTTTCTGACCAATAGATATTCATCAAAATGAACATTGGCAATTGCCAT
AAAAACGATAAATAACGTATTGGGATGTTGATTAATGATGAGCTTGATACGCTG
ACTGTTAGAAGCATCGTGGATGAAACAGTCCTCATTAATAAACACCACTGAAGG
GCGCTGTGAATCACAAGCTATGGCAAGGTCATCAACGGTTTCAATGTCGTTGATT
TCTCTTTTTTTAACCCCTCTACTCAACAGATACCCGGTTAAACCTAGTCGGGTGTA
ACTACATAAATCCATAATAATCGTTGACATGGCATACCCTCACTCAATGCGTAAC
GATAATTCCCCTTACCTGAATATTTCATCATGACTAAACGGAACAACATGGGTCA
CCTAATGCGCCACTCTCGCGATTTTTCAGGCGGACTTACTATCCCGTAAAGTGTT
GTATAATTTGCCTGGAATTGTCTTAAAGTAAAGTAAATGTTGCGATATGTGAGTG
AGCTTAAAACAAATATTTCGCTGCAGGAGTATCCTGGAAGATGTTCGTAGAAGCT
TACTGCTCACAAGAAAAAAGGCACGTCATCTGACGTGCCTTTTTTATTTGTACTA
CCCTGTACGATTACTGCAGCTCGAG**TTA**GGATTTCGTTTCGAATTGGGATTCGAT
TTTAACCCAGTCTTTGCACAGGATGTTTTCGTTACCGTAAATCCAGTGGGACGGA
CCAATGATAATTTTTTCCGGATTTTTGATCAGGTAGGCTGCCCACCAGGAGTAAG
TGCTGTTAGTGATGATACCGTGTTTGCAAGACTGCATCAGCATCATGTCCCAGTG
GGCTGCACCATCACGCGTCGTCATGTCAACAAACGGGTAACCCAGATCCAGGTTC
TGTACGAATTCCAGATCCTCGCAGAACAGGAACAGTTCCAGATTTTGAACACGTT
TTGCCATATACGCAATGGCGCGCAGCTGGTAGGAGATGTCCAGCTGCCAGCCCA
GGCGCATGTAATCGCCACGGCGGATGTGAACGAACACAGAGTTTTTCGCAGCCA
GGATCTGGGACAGTTTACGAGAGTACTGTTCCGCGTGTTCGGTCGGGTGAGGCAG
GGTGAAAGTTTGTTTGATCAGAGGGGAGATATCTTCGAAATAGCGCGGGTCCTG
AAAGTAGCCATGGAAATACGCAATGCGGCTCGGTTCAAACAGTTCCGGCATGTA
CTCGAATACAATTTCTTTGCTAACGCGGCCCAGACCCATACGACGCAGTGCACCA
CGCACCAGACGCGGCAGGTTCTGCATGTGTGCCGCGGCGATCTGCTGGGCGGAC
GCACACTGCAGGTCGATCGGGAACAGGTGCAGGCCCAGTTCACGGTTACCGTAA
TCGAACCAAGTGGTATCCAGCAGTACCGGAATGTTCAGGTGAGTCTGCAGAGAT

TTAGCGAATGCGTACTGGAACATCTGGTTACCCAGGCCGCCGTGCACCTGAACGA
TTTTGAAATCCATTATATCTCCTTCTTG

[0087] The nucleic acid sequence that encodes *H. mustelae* FutL protein from plasmid pG216 is set forth below (SEQ ID NO: 22) (the ATG start codon is underlined and the STOP codon is in bold and underlined):

ATGGATTTCAAAATCGTTCAGGTGCACGGCGGCCTGGGTAACCAGATGTTCCAGT
ACGCATTCGCTAAATCTCTGCAGACTCACCTGAACATTCCGGTACTGCTGGATAC
CACTTGGTTCGATTACGGTAACCGTGAACTGGGCCTGCACCTGTTCCCGATCGAC
CTGCAGTGTGCGTCCGCCCAGCAGATCGCCGCGGCACACATGCAGAACCTGCCG
CGTCTGGTGCGTGGTGCACTGCGTCGTATGGGTCTGGGCCGCGTTAGCAAAGAAA
TTGTATTCGAGTACATGCCGGAACTGTTTGAACCGAGCCGCATTGCGTATTTCCA
TGGCTACTTTCAGGACCCGCGCTATTTCGAAGATATCTCCCCTCTGATCAAACAA
ACTTTCACCCTGCCTCACCCGACCGAACACGCGGAACAGTACTCTCGTAAACTGT
CCCAGATCCTGGCTGCGAAAAACTCTGTGTTCGTTCACATCCGCCGTGGCGATTA
CATGCGCCTGGGCTGGCAGCTGGACATCTCCTACCAGCTGCGCGCCATTGCGTAT
ATGGCAAAACGTGTTCAAAATCTGGAACTGTTCCTGTTCTGCGAGGATCTGGAAT
TCGTACAGAACCTGGATCTGGGTTACCCGTTTGTTGACATGACGACGCGTGATGG
TGCAGCCCACTGGGACATGATGCTGATGCAGTCTTGCAAACACGGTATCATCACT
AACAGCACTTACTCCTGGTGGGCAGCCTACCTGATCAAAAATCCGGAAAAAATT
ATCATTGGTCCGTCCCACTGGATTTACGGTAACGAAAACATCCTGTGCAAAGACT
GGGTTAAAATCGAATCCCAATTCGAAACGAAATCC**TAA**

[0088] A map of plasmid pG217 is shown in Figure 10. The sequence of plasmid pG217 is set forth below (SEQ ID NO: 18) (the codon complementary to the 5' ATG start codon for *B. vulgatus FutN* is underlined, and the codon complementary to the STOP codon is in bold and underlined):

TCTAGAATTCTAAAAATTGATTGAATGTATGCAAATAAATGCATACACCATAGGT
GTGGTTTAATTTGATGCCCTTTTTCAGGGCTGGAATGTGTAAGAGCGGGGTTATT
TATGCTGTTGTTTTTTTGTTACTCGGGAAGGGCTTTACCTCTTCCGCATAAACGCT
TCCATCAGCGTTTATAGTTAAAAAAATCTTTCGGAACTGGTTTTGCGCTTACCCCA
ACCAACAGGGGATTTGCTGCTTTCCATTGAGCCTGTTTCTCTGCGCGACGTTCGC
GGCGGCGTGTTTGTGCATCCATCTGGATTCTCCTGTCAGTTAGCTTTGGTGGTGTG
TGGCAGTTGTAGTCCTGAACGAAAACCCCCCCGCGATTGGCACATTGGCAGCTAAT
CCGGAATCGCACTTACGGCCAATGCTTCGTTTCGTATCACACACCCCAAAGCCTT
CTGCTTTGAATGCTGCCCTTCTTCAGGGCTTAATTTTTAAGAGCGTCACCTTCATG
GTGGTCAGTGCGTCCTGCTGATGTGCTCAGTATCACCGCCAGTGGTATTTATGTC
AACACCGCCAGAGATAATTTATCACCGCAGATGGTTATCTGTATGTTTTTTATAT
GAATTTATTTTTTGCAGGGGGGCATTGTTTGGTAGGTGAGAGATCAATTCTGCAT
TAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCC
GCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTAT
CAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAG
GAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCC
GCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATC
GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGT
TTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGA

TACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTG
TAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAA
CCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA
ACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTA
GCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACT
ACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTAC
CTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAG
CGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAA
GAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCAC
GTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTT
AAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCT
GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTC
GTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG
CTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCT
CCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGT
CCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAG
TAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCAT
CGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGAT
CAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGG
TCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATG
GCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGAC
TGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGC
TCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAA
GTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGC
TGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATC
TTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA
AAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTC
AATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGA
ATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGT
GCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGG
CGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCT
GACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGA
GCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGC
TTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATATGCGGTGT
GAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCTCCTCAAC
CTGTATATTCGTAAACCACGCCCAATGGGAGCTGTCTCAGGTTTGTTCCTGATTG
GTTACGGCGCGTTTCGCATCATTGTTGAGTTTTTCCGCCAGCCCGACGCGCAGTTT
ACCGGTGCCTGGGTGCAGTACATCAGCATGGGGCAAATTCTTTCCATCCCGATGA
TTGTCGCGGGTGTGATCATGATGGTCTGGGCATATCGTCGCAGCCCACAGCAACA
CGTTTCCTGAGGAACCATGAAACAGTATTTAGAACTGATGCAAAAAGTGCTCGA
CGAAGGCACACAGAAAAACGACCGTACCGGAACCGGAACGCTTTCCATTTTTGG
TCATCAGATGCGTTTTAACCTGCAAGATGGATTCCCGCTGGTGACAACTAAACGT
TGCCACCTGCGTTCCATCATCCATGAACTGCTGTGGTTTCTGCAGGGCGACACTA
ACATTGCTTATCTACACGAAAACAATGTCACCATCTGGGACGAATGGGCCGATG
AAAACGGCGACCTCGGGCCAGTGTATGGTAAACAGTGGCGCGCCTGGCCAACGC
CAGATGGTCGTCATATTGACCAGATCACTACGGTACTGAACCAGCTGAAAAACG
ACCCGGATTCGCGCCGCATTATTGTTTCAGCGTGGAACGTAGGCGAACTGGATAA
AATGGCGCTGGCACCGTGCCATGCATTCTTCCAGTTCTATGTGGCAGACGGCAAA
CTCTCTTGCCAGCTTTATCAGCGCTCCTGTGACGTCTTCCTCGGCCTGCCGTTCAA
CATTGCCAGCTACGCGTTATTGGTGCATATGATGGCGCAGCAGTGCGATCTGGAA
GTGGGTGATTTTGTCTGGACCGGTGGCGACACGCATCTGTACAGCAACCATATGG

ATCAAACTCATCTGCAATTAAGCCGCGAACCGCGTCCGCTGCCGAAGTTGATTAT
CAAACGTAAACCCGAATCCATCTTCGACTACCGTTTCGAAGACTTTGAGATTGAA
GGCTACGATCCGCATCCGGGCATTAAAGCGCCGGTGGCTATCTAATTACGAAAC
ATCCTGCCAGAGCCGACGCCAGTGTGCGTCGGTTTTTTTACCCTCCGTTAAATTCT
TCGAGACGCCTTCCCGAAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGA
AGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATG
TGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGT
AAAACGACGGCCAGTGCCAAGCTTTCTTTAATGAAGCAGGGCATCAGGACGGTA
TCTTTGTGGAGAAAGCAGAGTAATCTTATTCAGCCTGACTGGTGGGAAACCACCA
GTCAGAATGTGTTAGCGCATGTTGACAAAAATACCATTAGTCACATTATCCGTCA
GTCGGACGACATGGTAGATAACCTGTTTATTATGCGTTTTGATCTTACGTTTAATA
TTACCTTTATGCGATGAAACGGTCTTGGCTTTGATATTCATTTGGTCAGAGATTTG
AATGGTTCCCTGACCTGCCATCCACATTCGCAACATACTCGATTCGGTTCGGCTC
AATGATAACGTCGGCATATTTAAAAACGAGGTTATCGTTGTCTCTTTTTTCAGAA
TATCGCCAAGGATATCGTCGAGAGATTCCGGTTTAATCGATTTAGAACTGATCAA
TAAATTTTTTCTGACCAATAGATATTCATCAAAATGAACATTGGCAATTGCCATA
AAAACGATAAATAACGTATTGGGATGTTGATTAATGATGAGCTTGATACGCTGAC
TGTTAGAAGCATCGTGGATGAAACAGTCCTCATTAATAAACACCACTGAAGGGC
GCTGTGAATCACAAGCTATGGCAAGGTCATCAACGGTTTCAATGTCGTTGATTTC
TCTTTTTTTAACCCCTCTACTCAACAGATACCCGGTTAAACCTAGTCGGGTGTAAC
TACATAAATCCATAATAATCGTTGACATGGCATACCCTCACTCAATGCGTAACGA
TAATTCCCCTTACCTGAATATTTCATCATGACTAAACGGAACAACATGGGTCACC
TAATGCGCCACTCTCGCGATTTTTCAGGCGGACTTACTATCCCGTAAAGTGTTGT
ATAATTTGCCTGGAATTGTCTTAAAGTAAAGTAAATGTTGCGATATGTGAGTGAG
CTTAAAACAAATATTTCGCTGCAGGAGTATCCTGGAAGATGTTCGTAGAAGCTTA
CTGCTCACAAGAAAAAAGGCACGTCATCTGACGTGCCTTTTTTATTTGTACTACC
CTGTACGATTACTGCAGCTCGAG**TTA**GGATACCGGCACTTTGATCCAACCAGTCG
GGTAGATATCCGGTGCTTCGGAGTGCTGGAACCAACGGCTCGGCACAATAACAG
TCTTATCCATATTAGGGTTCAGCCAGGCACCCCACCAAGAAAACGTGCTGTTACA
AATGATGTGATGTTTGCAATGAGACATCAGCATCATATCCTGCCAGGAGTCTTCA
TCAGTGTTCCAGTCAATATAAACCGCATTCTGCAGTGGCAGATTTTCTTTAACCC
ACGCGATATCGTCGGAGAAGATATAGTAAGATGGGCTAGCAACACGACGGGACA
TTTCCGCGATAGCATTCTGGTAATACGGCAGCTGGCACACGGAACCGGTAGTAGC
CCAGTGTTTCGGCTGCAGATAGTCACCACGACGAATGTGCAGGGAAACCGCGTTT
TCATCTTTGTCCAGGATTTCCAGCATGTTCAGGCTGCGGGAATTTGCTTTGTTCTT
ATCAAAGGTGAAGGATTCACGCACTTCGTCTTTGATATCAGCGAAGAAACGCTCG
CTCTGATAGAAACCTTTAAAGTACAGCAGCGGCCAGAAATACTTCTTCTCGAACG
CACGCAGAGAGTTCGGCGCCTGCTTGCGTTCGTAGATTTTTTTAAAAAACAGGAA
TTCGATAACTTTTTTCAGCGGTTGGTTGATGCAGAATTCGGTGTGCGGCAGGTTG
AACACGCGGTGCATTTCGTAACCGTAATGGACTTTGTAATGCATCATGTCGCTCA
GGTCGATACGGACCTTCGGGTAATACTTTTTCATACGCAGATAGAAAGCATAGAT
AAACATCTGGTTGCCCAGACCGCCAGTCACTTTGATCAGACG<u>CAT</u>TATATCTCCT
TCTTG

[0089] The nucleic acid sequence that encodes *B. vulgatus* FutN protein from plasmid pG217 is set forth below (SEQ ID NO: 23) (the ATG start codon is underlined and the STOP codon is in bold and underlined):

<u>ATG</u>CGTCTGATCAAAGTGACTGGCGGTCTGGGCAACCAGATGTTTATCTATGCTT
TCTATCTGCGTATGAAAAAGTATTACCCGAAGGTCCGTATCGACCTGAGCGACAT

GATGCATTACAAAGTCCATTACGGTTACGAAATGCACCGCGTGTTCAACCTGCCG
CACACCGAATTCTGCATCAACCAACCGCTGAAAAAAGTTATCGAATTCCTGTTTT
TTAAAAAAATCTACGAACGCAAGCAGGCGCCGAACTCTCTGCGTGCGTTCGAGA
AGAAGTATTTCTGGCCGCTGCTGTACTTTAAAGGTTTCTATCAGAGCGAGCGTTT
CTTCGCTGATATCAAAGACGAAGTGCGTGAATCCTTCACCTTTGATAAGAACAAA
GCAAATTCCCGCAGCCTGAACATGCTGGAAATCCTGGACAAAGATGAAAACGCG
GTTTCCCTGCACATTCGTCGTGGTGACTATCTGCAGCCGAAACACTGGGCTACTA
CCGGTTCCGTGTGCCAGCTGCCGTATTACCAGAATGCTATCGCGGAAATGTCCCG
TCGTGTTGCTAGCCCATCTTACTATATCTTCTCCGACGATATCGCGTGGGTTAAAG
AAAATCTGCCACTGCAGAATGCGGTTTATATTGACTGGAACACTGATGAAGACTC
CTGGCAGGATATGATGCTGATGTCTCATTGCAAACATCACATCATTTGTAACAGC
ACGTTTTCTTGGTGGGGTGCCTGGCTGAACCCTAATATGGATAAGACTGTTATTG
TGCCGAGCCGTTGGTTCCAGCACTCCGAAGCACCGGATATCTACCCGACTGGTTG
GATCAAAGTGCCGGTATCC**TAA**

[0090] Fucosylated oligosaccharides produced by metabolically engineered *E. coli* cells are purified from culture broth post-fermentation. An exemplary procedure comprises five steps. (1) Clarification: Fermentation broth is harvested and cells removed by sedimentation in a preparative centrifuge at 6000 x g for 30 min. Each bioreactor run yields about 5-7 L of partially clarified supernatant. (2) Product capture on coarse carbon : A column packed with coarse carbon (Calgon 12x40 TR) of ~1000 ml volume (dimension 5 cm diameter x 60 cm length) is equilibrated with 1 column volume (CV) of water and loaded with clarified culture supernatant at a flow rate of 40 ml/min. This column has a total capacity of about 120 g of sugar. Following loading and sugar capture, the column is washed with 1.5 CV of water, then eluted with 2.5 CV of 50% ethanol or 25% isopropanol (lower concentrations of ethanol at this step (25-30%) may be sufficient for product elution.) This solvent elution step releases about 95% of the total bound sugars on the column and a small portion of the color bodies. In this first step capture of the maximal amount of sugar is the primary objective. Resolution of contaminants is not an objective. (3) Evaporation: A volume of 2.5 L of ethanol or isopropanol eluate from the capture column is rotary-evaporated at 56 C° and a sugar syrup in water is generated. Alternative methods that could be used for this step include lyophilization or spray-drying. (4) Flash chromatography on fine carbon and ion exchange media: A column (GE Healthcare HiScale50/40, 5x40cm, max pressure 20 bar) connected to a Biotage Isolera One FLASH Chromatography System is packed with 750 ml of a Darco Activated Carbon G60 (100-mesh): Celite 535 (coarse) 1:1 mixture (both column packings were obtained from Sigma). The column is equilibrated with 5 CV of water and loaded with sugar from step 3 (10-50 g, depending on the ratio of 2'-FL to contaminating lactose), using either a celite loading cartridge or direct injection. The column is connected to an evaporative light scattering (ELSD) detector to detect peaks of eluting sugars during the chromatography. A four-step gradient of isopropanol, ethanol or methanol is run in order to separate 2'-FL from monosaccharides (if present), lactose and color bodies. Fractions corresponding to sugar peaks are collected automatically in 120-ml bottles, pooled and directed to step 5. In certain purification runs from longer-than-normal fermentations, passage of the 2'-FL-containing fraction through anion-exchange and cation exchange columns can remove excess protein/DNA/caramel body contaminants. Resins tested successfully for this purpose are Dowex 22

[0091] The identity of the major oligosaccharide synthesized by WbgL was tested and confirmed to be bona fide 2'-FI.. Oligosaccharides synthesized in the WbgL strain were immobilized on a carbon column, eluted and resuspended in distilled water. This material was subjected to overnight digestion with fucosidases of different specificities, and the reactions were analyzed by TLC. As shown in Figure 4, the untreated material consisted primarily of an oligosaccharide with the same mobility as the 2'-FL standard (lane 1). Treatment with α1,2 fucosidase yielded both lactose and fucose, while the presumptive 2'-FL spot was significantly diminished in staining intensity (lane 2). Treatment of the oligosaccharides with an α1,3-4 fucosidase had no effect. These results demonstrate that WbgL is capable of the biosynthesis of bona fide 2'-FL in metabolically engineered *E. coli.*

[0092] The gene screening approach was successfully utilized to identify new α(1,2) FTs for the efficient biosynthesis of 2'-FL in metabolically engineered *E. coli* host strains. The results of the screen are summarized in Table 1. Specifically, WbgL and FutL both direct the synthesis of 2'-FL at approximately 75% the levels attained by the previously characterized α(1,2) FT FutC. In addition, WbgL also was capable of synthesizing LDFT, which is another therapeutically useful HMO. Furthermore, FutN from the commensal enteric bacterium *B. vulgatus* was identified as another α(1,2) FT useful for the synthesis of fucosylated oligosaccharides. The approach described herein is useful in the analysis of additional candidate α(1,2) FTs and identifies additional enzymes that are useful for the large-scale production of HMOS.

Production Host Strains

[0093] *E. coli* K-12 is a well-studied bacterium which has been the subject of extensive research in microbial physiology and genetics and commercially exploited for a variety of industrial uses. The natural habitat of the parent species, *E. coli*, is the large bowel of mammals. *E. coli* K-12 has a history of safe use, and its derivatives are used in a large number of industrial applications, including the production of chemicals and drugs for human administration and consumption. *E. coli* K-12 was originally isolated from a convalescent diphtheria patient in 1922. Because it lacks virulence characteristics, grows readily on common laboratory media, and has been used extensively for microbial physiology and genetics research, it has become the standard bacteriological strain used in microbiological research, teaching, and production of products for industry and medicine. *E. coli* K-12 is now considered an enfeebled organism as a result of being maintained in the laboratory environment for over 70 years. As a result, K-12 strains are unable to colonize the intestines of humans and other animals under normal conditions. Additional information on this well known strain is available at http://epa.gov/oppt/biotech/pubs/fra/fra004.htm. In addition to *E. coli* K12, other bacterial strains are used as production host strains, e.g., a variety of bacterial species may be used in the oligosaccharide biosynthesis methods, e.g., *Erwinia herbicola* (*Pantoea agglomerans*), *Citrobacterfreundii*, *Pantoea citrea*, *Pectobacterium carotovorum*, *or Xanthomonas campestris.* Bacteria of the genus *Bacillus* may also be used, including *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus coagulans*, *Bacillus thermophilus*, *Bacillus laterosporus*, *Bacillus megaterium*, *Bacillus mycoides*, *Bacillus pumilus*, *Bacillus lentus*, *Bacillus cereus*, *and Bacillus circulans*. Similarly, bacteria of the genera *Lactobacillus* and *Lactococcus* may be modified using the methods of this invention, including but not limited to *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus jensenii, and Lactococcus lactis. Streptococcus thermophiles and Proprionibacterium freudenreichii* are also suitable bacterial species for the invention described herein. Also included as part of this invention are strains, modified as described here, from the genera *Enterococcus* (e.g., *Enterococcus faecium* and *Enterococcus thermophiles*), *Bifidobacterium* (e.g., *Bifidobacterium longum*, *Bifidobacterium infantis*, and *Bifidobacterium bifidum*), *Sporolactobacillus* spp., *Micromomospora* spp., *Micrococcus* spp., *Rhodococcus* spp., and *Pseudomonas* (e.g., *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*).

[0094] Suitable host strains are amenable to genetic manipulation, e.g., they maintain expression constructs, accumulate precursors of the desired end product, e.g., they maintain pools of lactose and GDP-fucose, and accumulate endproduct, e.g., 2'-FL. Such strains grow well on defined minimal media that contains simple salts and generally a single carbon source. The strains engineered as described above to produce the desired fucosylated oligosaccharide(s) are grown in a minimal media. An exemplary minimal medium used in a bioreactor, minimal "FERM" medium, is detailed below.

[0095] Ferm (10 liters): Minimal medium comprising:

40g $(NH_4)_2HPO_4$

100g KH_2PO_4

10g $MgSO_4.7H_2O$

40g NaOH

[0096] Trace elements:

1.3g NTA (nitrilotriacetic acid)

0. 5g $FeSO_4$ $.7H_2O$

0.09g $MnCl_2$ $.4H_2O$

0.09g $ZnSO_4$ $.7H_2O$

0.01g $CoCl_2$ $.6H_2O$

0.01g $CuCl_2$ $.2H_2O$

0.02g H_3BO_3

0.01g Na_2MoO_4 .$2H_2O$ (pH 6.8)

Water to 10 liters

DF204 antifoam (0.1ml/L)

150 g glycerol (initial batch growth), followed by fed batch mode with a 90% glycerol-1% $MgSO_4$-1X trace elements feed, at various rates for various times.

[0097] A suitable production host strain is one that is not the same bacterial strain as the source bacterial strain from which the fucosyltransferase-encoding nucleic acid sequence was identified. For example, the fucosyltransferase-encoding nucleic acid sequence FutL was identified in *Helicobacter mustelae* and a suitable host strain is a bacteria other than *Helicobacter mustelae*, e.g., FutL is produced in production host strain *E.coli* K12 or any of the other strains described above.

[0098] Bacteria comprising the characteristics described herein are cultured in the presence of lactose, and a fucosylated oligosaccharide is retrieved, either from the bacterium itself or from a culture supernatant of the bacterium. The fucosylated oligosaccharide is purified for use in therapeutic or nutritional products, or the bacteria are used directly in such products.

OTHER EMBODIMENTS

[0099] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

[0100] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

[0101] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

[0102] The following pages 57 to 62 contain specific embodiments.

1. A nucleic acid construct comprising an isolated nucleic acid encoding a lactose-utilizing α(1,2) fucosyltransferase enzyme, said nucleic acid being operably linked to one or more heterologous control sequences that direct the production of the enzyme in a host bacteria production strain, wherein the amino acid sequence of said enzyme encoded by said nucleic acid comprises at least 10% and less than 40% identity to SEQ ID NO:2.

2. The construct of 1, wherein said production strain comprises *Eschericia coli* K12.

3. The construct of 1, wherein said nucleic acid encodes a WbgL, FutL, or FutN protein,

4. The construct of 1, wherein said heterologous control sequence comprises a bacterial promoter and operator, a bacterial ribosome binding site, a bacterial transcriptional terminator, or a plasmid selectable marker.

5. The construct of 1, wherein said production strain is a member of the Bacillus genus.

6. The construct of 1, wherein said production strain comprises Bacillus licheniformis.

7. The construct of 1, wherein said production strain is a member of the Pantoea genus.

8. The construct of 1, wherein said production strain is a member of the Lactobacillus or Lactococcus genus.

9. The construct of 1, wherein said production strain is a member of the *Streptococcus, Proprionibacterium, Enterococcus, Bifidobacterium, Sporolactobacillus*, *Micromomospora*, *Micrococcus, Rhodococcus, or Pseudomonas* genus.

10. The construct of 1, wherein said production strain is selected from the group consisting of *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus thermophilus*, *Bacillus laterosporus*, *Bacillus megaterium*, *Bacillus mycoides, Bacillus pumilus*, *Bacillus lentus*, *Bacillus cereus*, *and Bacillus circulans*, *Erwinia herbicola* (*Pantoea agglomerans*), *Citrobacter freundii*, *Pantoea citrea*, *Pectobacterium carotovorum*, *Xanthomonas campestris Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus reuteri*, *Lactobacillus jensenii*, *Lactococcus lactis*, *Streptococcus thermophiles*, *Proprionibacterium freudenreichii*, *Enterococcus faecium*, *Enterococcus thermophiles*), *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium bifidum*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*.

11. An *E.coli* cell, *E.coli* culture supernatant, or *E.coli* cell lysate comprising 2'-FL, wherein said cell, supernatant, or lysate does not substantially comprise a 1,3 fucosylated lactose prior to purification of 2'-FL from said cell, culture supernatant, or lysate.

12. An isolated *Escherichia coli* (*E. coli*) bacterium comprising a reduced level of β-galactosidase activity, a defective colonic acid synthesis pathway, a mutation in an adenosine-5'-triphosphate (ATP)-dependant intracellular protease, a mutation in the *lacA* gene, a mutation in the *thyA* gene, and an isolated nucleic acid encoding a lactose-accepting α (1,2) fucosyltransferase enzyme, said nucleic acid, wherein the sequence of said nucleic acid comprises at least 10% and less than 40% identity to SEQ ID NO:2.

13. The isolated *E. coli* bacterium of 12, wherein an endogenous *lacZ* gene and an endogenous /*acI* gene of said *E. coli* bacterium are deleted or functionally inactivated.

14. The isolated *E. coli* bacterium of 13, wherein said *E. coli* bacterium comprises a *lucIq* gene promoter upstream of a *lacY* gene.

15. The isolated *E. coli* bacterium of 12, wherein an endogenous *wcaJ* gene of said *E. coli* bacterium is deleted or functionally inactivated.

16. The isolated *E. coli* bacterium of 12, wherein said mutation in said ATP-dependant intracellular protease is a mutation in a *lon* gene.

17. The isolated *E. coli* bacterium of 12, wherein said bacterium accumulates intracellular lactose in the presence of exogenous lactose.

18. The isolated *E. coli* bacterium of 12, wherein said bacterium accumulates intracellular guanosine diphosphate (GDP)-fucose.

19. The isolated *E. coli* bacterium of 12, wherein said exogenous α (1,2) fucosyltransferase gene comprises at least 25% identity to *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (*futC*).

20. The isolated *E. coli* bacterium of 12, wherein said exogenous α (1,2) fucosyltransferase gene is selected from the group consisting of, *Vibrio cholera* O22 *wblA*, *Escherichia coli* O126 *wbgL*, *Helicobacter bilis* ATCC 437879 *futD*, *Helicobacter cinaedi* CCUG 18818 alpha-1,2-fucosyltransferase (*futE*), *Helicobacter mustelae* 12198 (ATCC 43772) alpha-1,2-fucosyltransferase (*futL*), *Bacteroides vulgatus* ATCC 8482 glycosyl transferase family protein (*futN*), *Bacteroides ovatus* ATCC 8483 *futO*, *Escherichia coli* O55:H7 (str. CB9615) fucosyltransferase (*wbgN*), *Bacteroides fragilis* (NCTC) 9343 alpha-1,2-fucosyltransferase (*bft1*), and *Bacteroides fragilis* (NCTC) 9343 fucosyl transferase (*bft3*/*wcfB*).

21. The isolated *E. coli* bacterium of 12, wherein said *E. coli* bacterium comprises the genotype Δ*ampC*::$P_{trp}{}^{B}$*cI*, Δ(*lacI-lacZ*)::*FRT*, P_{laclq}*lacY*$^{+}$, Δ*wcaJ*::FRT, *thyA*::Tn*10*, Δ*lon*:(*npt3*, *lacZ*$^{+}$).

22. A nucleic acid construct comprising an isolated nucleic acid encoding a lactose-utilizing α (1,2) fucosyltransferase enzyme, said nucleic acid being operably linked to one or more heterologous control heterologous sequences that direct the production of the enzyme in a host bacteria production strain, wherein the amino acid sequence of said enzyme encoded by the sequence of said nucleic acid comprises about 70% identity to SEQ ID NO:2.

23. The nucleic acid construct of 22, wherein said construct comprises SEQ ID NO: 7.

24. A method for producing a fucosylated oligosaccharide in a bacterium comprising providing bacterium comprising a reduced level of β-galactosidase activity, a defective colonic acid synthesis pathway, a mutation in an ATP-dependant intracellular protease, a mutation in a *thyA* gene, and an exogenous lactose-accepting α (1,2) fucosyltransferase gene; culturing said bacterium in the presence of lactose; retrieving a fucosylated oligosaccharide from said bacterium or from a culture supernatant of said bacterium.

25. The method of 24, wherein said enteric bacterium comprises *E. coli.*

26. The method of 24, wherein said fucosylated oligosaccharide comprises 2'-fucosyllactose (2'-FL) or lactodifucotetraose (LDFT), Lacto-N-fucopentaose I (LNF I), or lacto-N-difucohexaose I (LDFH I).

27. The method of 24, wherein said method further comprises culturing said bacterium in the presence of tryptophan and in the absence of thymidine.

28. The method of 24, wherein an endogenous *lacZ* gene and an endogenous *lacI* gene of said *E. coli* are deleted.

29. The method of 28, wherein said *E. coli* bacterium comprises a *lacIq* gene promoter immediately upstream of a *lacY* gene.

30. The method of 24, wherein an endogenous *wcaJ* gene of said *E. coli* is deleted.

31. The method of 24, wherein said mutation in said ATP-dependant intracellular protease is a null mutation in a *lon* gene.

32. The method of 24, wherein said bacterium accumulates intracellular lactose in the presence of exogenous lactose.

33. The method of 24, wherein said bacterium accumulates intracellular GDP-fucose.

34. The method of 24, wherein said α (1,2) fucosyltransferase gene comprises at least 25% homology or identity to *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (*futC*).

35. The method of 28, wherein said wherein said α (1,2) fucosyltransferase gene is selected from the group consisting of *Helicobacter pylori* 26695 alpha-(1,2) fucosyltransferase (*futC*), *Vibrio cholera* O22 *wblA*, *Escherichia coli* O126 *wbgL*, *Helicobacter bilis* ATCC 437879 *futD*, *Helicobacter cinaedi* CCUG 18818 alpha-1,2-fucosyltransferase (*futE*), *Helicobacter mustelae* 12198 (ATCC 43772) alpha-1,2-fucosyltransferase (*futL*), *Bacteroides vulgatus* ATCC 8482 glycosyl transferase family protein (*futN*), *Bacteroides ovatus* ATCC 8483 *futO*, *Escherichia coli* O55:H7 (str. CB9615) fucosyltransferase (*wbgN*), *Bacteroides fragilis* (NCTC) 9343 alpha-1,2-fucosyltransferase (*bft1*), and *Bacteroides fragilis* (NCTC) 9343 fucosyl transferase (*bft3*/*wcfB*).

36. A purified fucosylated oligosaccharide produced by the method of 24.

37. A method of identifying non-FutC lactose-utilizing, α(1,2)fucosyltransferase enzyme comprising the following steps:

1) performing a computational search of sequence databases to define a broad group of simple sequence homologs of any known, lactose-utilizing α(1,2)fucosyltransferase;
2) using the list from step (1), deriving a search profile containing common sequence and/or structural motifs shared by the members of the list;
3) searching sequence databases, using a derived search profile based on the common sequence or structural motif from step (2) as query, and identifying a candidate sequences, wherein a sequence homology to a reference lactose-utilizing α(1,2)fucosyltransferase is 40% or less;
4) compiling a list of candidate organisms, said organisms being characterized as expressing α(1,2)fucosylglycans in a naturally-occurring state;
5) selecting candidate sequences that are derived from candidate organisms to generate a list of candidate lactose-utilizing enzymes;
6) expressing the candidate lactose- utilizing enzyme in a host organism; and
7) testing for lactose- utilizing α(1,2)fucosyltransferase activity, wherein detection of 2'-FL in said organism indicates that the candidate sequence comprises a non-FutC lactose-utilizing α(1,2)fucosyltransferase.

38. The method of 37, wherein a sequence homology to a reference lactose-utilizing α(1,2)fucosyltransferase is 40% or less.

SEQUENCE LISTING

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catagcagaa ctttaaaagt gctcatcatt ggaaaacgtt cttcggggcg aaaactctca 5880

aggatcttac cgctgttgag atccagttcg atgtaaccca ctcgtgcacc caactgatct 5940

tcagcatctt ttactttcac agcgtttct gggtgagcaa aaacaggaag gcaaaatgcc 6000

gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt cctttttcaa 6060

tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt tgaatgtatt 6120

tagaaaaata aacaaatagg ggttccgcgc acatttcccc gaaaagtgcc acctgacgtc 6180

taagaaacca ttattatcat gacattaacc tataaaaata ggcgtatcac gaggcccttt 6240

cgtc 6244

<210> 2
<211> 298
<212> PRT
<213> Helicobacter pylori

<400> 2

Met Ala Phe Lys Val Val Gln Ile Cys Gly Gly Leu Gly Asn Gln Met
1 5 10 15

Phe Gln Tyr Ala Phe Ala Lys Ser Leu Gln Lys His Leu Asn Thr Pro
20 25 30

```
Val Leu Leu Asp Ile Thr Ser Phe Asp Trp Ser Asn Arg Lys Met Gln
        35                  40                  45

Leu Glu Leu Phe Pro Ile Asp Leu Pro Tyr Ala Ser Ala Lys Glu Ile
    50                  55                  60

Ala Ile Ala Lys Met Gln His Leu Pro Lys Leu Val Arg Asp Thr Leu
65                  70                  75                  80

Lys Cys Met Gly Phe Asp Arg Val Ser Gln Glu Ile Val Phe Glu Tyr
                85                  90                  95

Glu Pro Gly Leu Leu Lys Pro Ser Arg Leu Thr Tyr Phe Tyr Gly Tyr
            100                 105                 110

Phe Gln Asp Pro Arg Tyr Phe Asp Ala Ile Ser Pro Leu Ile Lys Gln
        115                 120                 125

Thr Phe Thr Leu Pro Pro Pro Glu Asn Gly Asn Asn Lys Lys Lys Glu
    130                 135                 140

Glu Glu Tyr His Arg Lys Leu Ala Leu Ile Leu Ala Ala Lys Asn Ser
145                 150                 155                 160

Val Phe Val His Val Arg Arg Gly Asp Tyr Val Gly Ile Gly Cys Gln
                165                 170                 175

Leu Gly Ile Asp Tyr Gln Lys Lys Ala Leu Glu Tyr Ile Ala Lys Arg
            180                 185                 190

Val Pro Asn Met Glu Leu Phe Val Phe Cys Glu Asp Leu Lys Phe Thr
        195                 200                 205

Gln Asn Leu Asp Leu Gly Tyr Pro Phe Met Asp Met Thr Thr Arg Asp
    210                 215                 220

Lys Glu Glu Glu Ala Tyr Trp Asp Met Leu Leu Met Gln Ser Cys Lys
225                 230                 235                 240

His Gly Ile Ile Ala Asn Ser Thr Tyr Ser Trp Trp Ala Ala Tyr Leu
                245                 250                 255

Ile Asn Asn Pro Glu Lys Ile Ile Ile Gly Pro Lys His Trp Leu Phe
            260                 265                 270

Gly His Glu Asn Ile Leu Cys Lys Glu Trp Val Lys Ile Glu Ser His
```

275 280 285

Phe Glu Val Lys Ser Lys Lys Tyr Asn Ala
290 295

<210> 3
<211> 281
<212> PRT
<213> Vibrio cholerae

<400> 3

Met Ile Val Met Lys Ile Ser Gly Gly Leu Gly Asn Gln Leu Phe Gln
1 5 10 15

Tyr Ala Val Gly Arg Ala Ile Ala Ile Gln Tyr Gly Val Pro Leu Lys
20 25 30

Leu Asp Val Ser Ala Tyr Lys Asn Tyr Lys Leu His Asn Gly Tyr Arg
35 40 45

Leu Asp Gln Phe Asn Ile Asn Ala Asp Ile Ala Asn Glu Asp Glu Ile
50 55 60

Phe His Leu Lys Gly Ser Ser Asn Arg Leu Ser Arg Ile Leu Arg Arg
65 70 75 80

Leu Gly Trp Leu Lys Lys Asn Thr Tyr Tyr Ala Glu Lys Gln Arg Thr
85 90 95

Ile Tyr Asp Val Ser Val Phe Met Gln Ala Pro Arg Tyr Leu Asp Gly
100 105 110

Tyr Trp Gln Asn Glu Gln Tyr Phe Ser Gln Ile Arg Ala Val Leu Leu
115 120 125

Gln Glu Leu Trp Pro Asn Gln Pro Leu Ser Ile Asn Ala Gln Ala His
130 135 140

Gln Ile Lys Ile Gln Gln Thr His Ala Val Ser Ile His Val Arg Arg
145 150 155 160

Gly Asp Tyr Leu Asn His Pro Glu Ile Gly Val Leu Asp Ile Asp Tyr
165 170 175

Tyr Lys Arg Ala Val Asp Tyr Ile Lys Glu Lys Ile Glu Ala Pro Val
180 185 190

Phe Phe Val Phe Ser Asn Asp Val Ala Trp Cys Lys Asp Asn Phe Asn

195 200 205

Phe Ile Asp Ser Pro Val Phe Ile Glu Asp Thr Gln Thr Glu Ile Asp
210 215 220

Asp Leu Met Leu Met Cys Gln Cys Gln His Asn Ile Val Ala Asn Ser
225 230 235 240

Ser Phe Ser Trp Trp Ala Ala Trp Leu Asn Ser Asn Val Asp Lys Ile
245 250 255

Val Ile Ala Pro Lys Thr Trp Met Ala Glu Asn Pro Lys Gly Tyr Lys
260 265 270

Trp Val Pro Asp Ser Trp Arg Glu Ile
275 280

<210> 4
<211> 297
<212> PRT
<213> Escherichia coli

<400> 4

Met Ser Ile Ile Arg Leu Gln Gly Gly Leu Gly Asn Gln Leu Phe Gln
1 5 10 15

Phe Ser Phe Gly Tyr Ala Leu Ser Lys Ile Asn Gly Thr Pro Leu Tyr
20 25 30

Phe Asp Ile Ser His Tyr Ala Glu Asn Asp Asp His Gly Gly Tyr Arg
35 40 45

Leu Asn Asn Leu Gln Ile Pro Glu Glu Tyr Leu Gln Tyr Tyr Thr Pro
50 55 60

Lys Ile Asn Asn Ile Tyr Lys Phe Leu Val Arg Gly Ser Arg Leu Tyr
65 70 75 80

Pro Glu Ile Phe Leu Phe Leu Gly Phe Cys Asn Glu Phe His Ala Tyr
85 90 95

Gly Tyr Asp Phe Glu Tyr Ile Ala Gln Lys Trp Lys Ser Lys Lys Tyr
100 105 110

Ile Gly Tyr Trp Gln Ser Glu His Phe Phe His Lys His Ile Leu Asp
115 120 125

Leu Lys Glu Phe Phe Ile Pro Lys Asn Val Ser Glu Gln Ala Asn Leu

```
    130                 135                 140

Leu Ala Ala Lys Ile Leu Glu Ser Gln Ser Ser Leu Ser Ile His Ile
145                 150                 155                 160

Arg Arg Gly Asp Tyr Ile Lys Asn Lys Thr Ala Thr Leu Thr His Gly
                165                 170                 175

Val Cys Ser Leu Glu Tyr Tyr Lys Lys Ala Leu Asn Lys Ile Arg Asp
            180                 185                 190

Leu Ala Met Ile Arg Asp Val Phe Ile Phe Ser Asp Asp Ile Phe Trp
        195                 200                 205

Cys Lys Glu Asn Ile Glu Thr Leu Leu Ser Lys Lys Tyr Asn Ile Tyr
    210                 215                 220

Tyr Ser Glu Asp Leu Ser Gln Glu Glu Asp Leu Trp Leu Met Ser Leu
225                 230                 235                 240

Ala Asn His His Ile Ile Ala Asn Ser Ser Phe Ser Trp Trp Gly Ala
                245                 250                 255

Tyr Leu Gly Thr Ser Ala Ser Gln Ile Val Ile Tyr Pro Thr Pro Trp
            260                 265                 270

Tyr Asp Ile Thr Pro Lys Asn Thr Tyr Ile Pro Ile Val Asn His Trp
        275                 280                 285

Ile Asn Val Asp Lys His Ser Ser Cys
    290                 295
```

<210> 5
<211> 293
<212> PRT
<213> Helicobacter bilis

<400> 5

```
Met Gly Asp Tyr Lys Ile Val Glu Leu Thr Cys Gly Leu Gly Asn Gln
1               5                   10                  15

Met Phe Gln Tyr Ala Phe Ala Lys Ala Leu Gln Lys His Leu Gln Val
            20                  25                  30

Pro Val Leu Leu Asp Lys Thr Trp Tyr Asp Thr Gln Asp Asn Ser Thr
        35                  40                  45

Gln Phe Ser Leu Asp Ile Phe Asn Val Asp Leu Glu Tyr Ala Thr Asn
```

```
     50                  55                  60

Thr Gln Ile Glu Lys Ala Lys Ala Arg Val Ser Lys Leu Pro Gly Leu
65                  70                  75                  80

Leu Arg Lys Met Phe Gly Leu Lys Lys His Asn Ile Ala Tyr Ser Gln
                85                  90                  95

Ser Phe Asp Phe His Asp Glu Tyr Leu Leu Pro Asn Asp Phe Thr Tyr
            100                 105                 110

Phe Ser Gly Phe Phe Gln Asn Ala Lys Tyr Leu Lys Gly Leu Glu Gln
        115                 120                 125

Glu Leu Lys Ser Ile Phe Tyr Tyr Asp Ser Asn Asn Phe Ser Asn Phe
    130                 135                 140

Gly Lys Gln Arg Leu Glu Leu Ile Leu Gln Ala Lys Asn Ser Ile Phe
145                 150                 155                 160

Ile His Ile Arg Arg Gly Asp Tyr Cys Lys Ile Gly Trp Glu Leu Gly
                165                 170                 175

Met Asp Tyr Tyr Lys Arg Ala Ile Gln Tyr Ile Met Asp Arg Val Glu
            180                 185                 190

Glu Pro Lys Phe Phe Ile Phe Gly Ala Thr Asp Met Ser Phe Thr Glu
        195                 200                 205

Gln Phe Gln Lys Asn Leu Gly Leu Asn Glu Asn Asn Ser Ala Asn Leu
    210                 215                 220

Ser Glu Lys Thr Ile Thr Gln Asp Asn Gln His Glu Asp Met Phe Leu
225                 230                 235                 240

Met Cys Tyr Cys Lys His Ala Ile Leu Ala Asn Ser Ser Tyr Ser Phe
                245                 250                 255

Trp Ser Ala Tyr Leu Asn Asn Asp Ala Asn Asn Ile Val Ile Ala Pro
            260                 265                 270

Thr Pro Trp Leu Leu Asp Asn Asp Asn Ile Ile Cys Asp Asp Trp Ile
        275                 280                 285

Lys Ile Ser Ser Lys
    290
```

<210> 6
<211> 257
<212> PRT
<213> Helicobacter cinaedi

<400> 6

Met Leu Phe Pro Phe Lys Phe Ile Tyr Asn Arg Leu Arg Tyr Lys Ala
1 5 10 15

Ile Arg Leu Ile Arg Arg Arg Ala Ser Tyr Arg Pro Phe Tyr Glu Phe
20 25 30

Tyr Ala His Ile Val Trp Gly Glu Glu Gly Val Val Asn Asp Arg Ile
35 40 45

Met Lys His Tyr Arg Glu Ser Ser Phe Lys Pro Tyr Ala Phe Pro Tyr
50 55 60

Gly Ile Asn Met Ser Phe Val Tyr Ser Asn Asp Val Tyr Ala Leu Leu
65 70 75 80

Lys Asp Asp Phe Arg Leu Lys Ile Pro Leu Arg Tyr Asp Asn Ala Met
85 90 95

Leu Lys Lys Gln Ile Gln Asn Thr Asp Lys Ser Val Phe Leu His Ile
100 105 110

Arg Arg Gly Asp Tyr Leu Gln Ser Glu Gly Leu Tyr Val Val Leu Gly
115 120 125

Val Thr Tyr Tyr Gln Lys Ala Leu Glu Ile Leu Lys Ser Lys Ile Thr
130 135 140

Asn Pro His Ile Phe Val Phe Ser Asn Asp Met Cys Trp Cys Lys Glu
145 150 155 160

Tyr Leu Met Arg Tyr Val Asp Phe Ser Gly Cys Thr Ile Asp Phe Ile
165 170 175

Glu Gly Asn Thr Glu Gly Asn Ala Val Glu Glu Met Glu Leu Met Arg
180 185 190

Ser Cys Gln His Ala Ile Ile Ala Asn Ser Thr Phe Ser Trp Trp Ala
195 200 205

Ala Tyr Leu Ile Glu Asn Pro Asp Lys Ile Val Ile Met Pro Lys Glu
210 215 220

Tyr Leu Asn Asp Ser Ser Arg Phe Leu Pro Lys Gln Phe Leu Ala Leu
225 230 235 240

Lys Asn Trp Phe Leu Val Asp His Ile Trp Gly Ser Val Glu Leu Ala
245 250 255

Asn

<210> 7
<211> 286
<212> PRT
<213> Helicobacter mustelae

<400> 7

Met Asp Phe Lys Ile Val Gln Val His Gly Gly Leu Gly Asn Gln Met
1 5 10 15

Phe Gln Tyr Ala Phe Ala Lys Ser Leu Gln Thr His Leu Asn Ile Pro
20 25 30

Val Leu Leu Asp Thr Thr Trp Phe Asp Tyr Gly Asn Arg Glu Leu Gly
35 40 45

Leu His Leu Phe Pro Ile Asp Leu Gln Cys Ala Ser Ala Gln Gln Ile
50 55 60

Ala Ala Ala His Met Gln Asn Leu Pro Arg Leu Val Arg Gly Ala Leu
65 70 75 80

Arg Arg Met Gly Leu Gly Arg Val Ser Lys Glu Ile Val Phe Glu Tyr
85 90 95

Met Pro Glu Leu Phe Glu Pro Ser Arg Ile Ala Tyr Phe His Gly Tyr
100 105 110

Phe Gln Asp Pro Arg Tyr Phe Glu Asp Ile Ser Pro Leu Ile Lys Gln
115 120 125

Thr Phe Thr Leu Pro His Pro Thr Glu His Ala Glu Gln Tyr Ser Arg
130 135 140

Lys Leu Ser Gln Ile Leu Ala Ala Lys Asn Ser Val Phe Val His Ile
145 150 155 160

Arg Arg Gly Asp Tyr Met Arg Leu Gly Trp Gln Leu Asp Ile Ser Tyr
165 170 175

```
Gln Leu Arg Ala Ile Ala Tyr Met Ala Lys Arg Val Gln Asn Leu Glu
            180                 185                 190

Leu Phe Leu Phe Cys Glu Asp Leu Glu Phe Val Gln Asn Leu Asp Leu
        195                 200                 205

Gly Tyr Pro Phe Val Asp Met Thr Thr Arg Asp Gly Ala Ala His Trp
    210                 215                 220

Asp Met Met Leu Met Gln Ser Cys Lys His Gly Ile Ile Thr Asn Ser
225                 230                 235                 240

Thr Tyr Ser Trp Trp Ala Ala Tyr Leu Ile Lys Asn Pro Glu Lys Ile
                245                 250                 255

Ile Ile Gly Pro Ser His Trp Ile Tyr Gly Asn Glu Asn Ile Leu Cys
            260                 265                 270

Lys Asp Trp Val Lys Ile Glu Ser Gln Phe Glu Thr Lys Ser
        275                 280                 285
```

<210> 8
<211> 281
<212> PRT
<213> Bacteroides vulgatus

<400> 8

```
Met Arg Leu Ile Lys Val Thr Gly Gly Leu Gly Asn Gln Met Phe Ile
1               5                   10                  15

Tyr Ala Phe Tyr Leu Arg Met Lys Lys Tyr Tyr Pro Lys Val Arg Ile
            20                  25                  30

Asp Leu Ser Asp Met Met His Tyr Lys Val His Tyr Gly Tyr Glu Met
        35                  40                  45

His Arg Val Phe Asn Leu Pro His Thr Glu Phe Cys Ile Asn Gln Pro
    50                  55                  60

Leu Lys Lys Val Ile Glu Phe Leu Phe Phe Lys Lys Ile Tyr Glu Arg
65                  70                  75                  80

Lys Gln Ala Pro Asn Ser Leu Arg Ala Phe Glu Lys Lys Tyr Phe Trp
                85                  90                  95

Pro Leu Leu Tyr Phe Lys Gly Phe Tyr Gln Ser Glu Arg Phe Phe Ala
            100                 105                 110
```

Asp Ile Lys Asp Glu Val Arg Glu Ser Phe Thr Phe Asp Lys Asn Lys
115 120 125

Ala Asn Ser Arg Ser Leu Asn Met Leu Glu Ile Leu Asp Lys Asp Glu
130 135 140

Asn Ala Val Ser Leu His Ile Arg Arg Gly Asp Tyr Leu Gln Pro Lys
145 150 155 160

His Trp Ala Thr Thr Gly Ser Val Cys Gln Leu Pro Tyr Tyr Gln Asn
165 170 175

Ala Ile Ala Glu Met Ser Arg Arg Val Ala Ser Pro Ser Tyr Tyr Ile
180 185 190

Phe Ser Asp Asp Ile Ala Trp Val Lys Glu Asn Leu Pro Leu Gln Asn
195 200 205

Ala Val Tyr Ile Asp Trp Asn Thr Asp Glu Asp Ser Trp Gln Asp Met
210 215 220

Met Leu Met Ser His Cys Lys His His Ile Ile Cys Asn Ser Thr Phe
225 230 235 240

Ser Trp Trp Gly Ala Trp Leu Asn Pro Asn Met Asp Lys Thr Val Ile
245 250 255

Val Pro Ser Arg Trp Phe Gln His Ser Glu Ala Pro Asp Ile Tyr Pro
260 265 270

Thr Gly Trp Ile Lys Val Pro Val Ser
275 280

<210> 9
<211> 292
<212> PRT
<213> Bacteroides ovatus

<400> 9

Met Lys Ile Val Asn Ile Leu Gly Gly Leu Gly Asn Gln Met Phe Val
1 5 10 15

Tyr Ala Met Tyr Leu Ala Leu Lys Glu Ala His Pro Glu Glu Glu Ile
20 25 30

Leu Leu Cys Arg Arg Ser Tyr Lys Gly Tyr Pro Leu His Asn Gly Tyr
35 40 45

```
Glu Leu Glu Arg Ile Phe Gly Val Glu Ala Pro Glu Ala Ala Leu Ser
    50                  55                  60

Gln Leu Ala Arg Val Ala Tyr Pro Phe Phe Asn Tyr Lys Ser Trp Gln
65                  70                  75                  80

Leu Met Arg His Phe Leu Pro Leu Arg Lys Ser Met Ala Ser Gly Thr
                85                  90                  95

Thr Gln Ile Pro Phe Asp Tyr Ser Glu Val Thr Arg Asn Asp Asn Val
            100                 105                 110

Tyr Tyr Asp Gly Tyr Trp Gln Asn Glu Lys Asn Phe Leu Ser Ile Arg
        115                 120                 125

Asp Lys Val Ile Lys Ala Phe Thr Phe Pro Glu Phe Arg Asp Glu Lys
    130                 135                 140

Asn Lys Ala Leu Ser Asp Lys Leu Lys Ser Val Lys Thr Ala Ser Cys
145                 150                 155                 160

His Ile Arg Arg Gly Asp Tyr Leu Lys Asp Pro Ile Tyr Gly Val Cys
                165                 170                 175

Asn Ser Asp Tyr Tyr Thr Arg Ala Ile Thr Glu Leu Asn Gln Ser Val
            180                 185                 190

Asn Pro Asp Met Tyr Cys Ile Phe Ser Asp Asp Ile Gly Trp Cys Lys
        195                 200                 205

Glu Asn Phe Lys Phe Leu Ile Gly Asp Lys Glu Val Val Phe Val Asp
    210                 215                 220

Trp Asn Lys Gly Gln Glu Ser Phe Tyr Asp Met Gln Leu Met Ser Leu
225                 230                 235                 240

Cys His Tyr Asn Ile Ile Ala Asn Ser Ser Phe Ser Trp Trp Gly Ala
                245                 250                 255

Trp Leu Asn Asn Asn Asp Asp Lys Val Val Val Ala Pro Glu Arg Trp
            260                 265                 270

Met Asn Lys Thr Leu Glu Asn Asp Pro Ile Cys Asp Asn Trp Lys Arg
        275                 280                 285

Ile Lys Val Glu
    290
```

<210> 10
<211> 290
<212> PRT
<213> Escherichia coli

<400> 10

Met Ser Ile Val Val Ala Arg Leu Ala Gly Gly Leu Gly Asn Gln Met
1 5 10 15

Phe Gln Tyr Ala Lys Gly Tyr Ala Glu Ser Val Glu Arg Asn Ser Ser
20 25 30

Leu Lys Leu Asp Leu Arg Gly Tyr Lys Asn Tyr Thr Leu His Gly Gly
35 40 45

Phe Arg Leu Asp Lys Leu Asn Ile Asp Asn Thr Phe Val Met Ser Lys
50 55 60

Lys Glu Met Cys Ile Phe Pro Asn Phe Ile Val Arg Ala Ile Asn Lys
65 70 75 80

Phe Pro Lys Leu Ser Leu Cys Ser Lys Arg Phe Glu Ser Glu Gln Tyr
85 90 95

Ser Lys Lys Ile Asn Gly Ser Met Lys Gly Ser Val Glu Phe Ile Gly
100 105 110

Phe Trp Gln Asn Glu Arg Tyr Phe Leu Glu His Lys Glu Lys Leu Arg
115 120 125

Glu Ile Phe Thr Pro Ile Asn Ile Asn Leu Asp Ala Lys Glu Leu Ser
130 135 140

Asp Val Ile Arg Cys Thr Asn Ser Val Ser Val His Ile Arg Arg Gly
145 150 155 160

Asp Tyr Val Ser Asn Val Glu Ala Leu Lys Ile His Gly Leu Cys Thr
165 170 175

Glu Arg Tyr Tyr Ile Asp Ser Ile Arg Tyr Leu Lys Glu Arg Phe Asn
180 185 190

Asn Leu Val Phe Phe Val Phe Ser Asp Asp Ile Glu Trp Cys Lys Lys
195 200 205

Tyr Lys Asn Glu Ile Phe Ser Arg Ser Asp Asp Val Lys Phe Ile Glu
210 215 220

Gly Asn Thr Gln Glu Val Asp Met Trp Leu Met Ser Asn Ala Lys Tyr
225 230 235 240

His Ile Ile Ala Asn Ser Ser Phe Ser Trp Trp Gly Ala Trp Leu Lys
245 250 255

Asn Tyr Asp Leu Gly Ile Thr Ile Ala Pro Thr Pro Trp Phe Glu Arg
260 265 270

Glu Glu Leu Asn Ser Phe Asp Pro Cys Pro Glu Lys Trp Val Arg Ile
275 280 285

Glu Lys
290

<210> 11
<211> 289
<212> PRT
<213> Bacteroides fragilis

<400> 11

Met Phe Phe Arg Cys Cys Met Lys Ile Val Gln Ile Ile Gly Gly Leu
1 5 10 15

Gly Asn Gln Met Phe Gln Phe Ala Phe Tyr Leu Ala Leu Lys Glu Lys
20 25 30

Tyr Val Asn Val Lys Leu Asp Thr Ser Ser Phe Gly Ala Tyr Thr His
35 40 45

Asn Gly Phe Glu Leu Asp Lys Val Phe His Val Glu Tyr Leu Lys Ala
50 55 60

Ser Ile Arg Glu Arg Ile Lys Leu Ser Tyr Gln Gly Ser Glu Ile Trp
65 70 75 80

Ile Arg Val Leu Arg Lys Leu Leu Lys Arg Lys Lys Thr Glu Tyr Val
85 90 95

Glu Pro Tyr Leu Cys Phe Asp Glu Asn Ala Ile Ser Leu Ser Cys Asp
100 105 110

Lys Tyr Tyr Ile Gly Tyr Trp Gln Ser Tyr Lys Tyr Phe Thr Asn Ile
115 120 125

Glu Ala Ala Ile Arg Gly Gln Phe His Phe Ser Lys Val Leu Ser Asp
130 135 140

```
Lys Asn Glu Phe Ile Lys Lys Gln Met Gln Asn Ser Asn Ser Val Ser
145                 150                 155                 160

Leu His Val Arg Leu Gly Asp Tyr Val Asn Asn Pro Ala Tyr Ser Asn
                165                 170                 175

Ile Cys Thr Ser Ala Tyr Tyr Asn Lys Ala Ile Asn Ile Ile Gln Ser
            180                 185                 190

Lys Val Ser Glu Pro Lys Phe Phe Val Phe Ser Asp Asp Thr Val Trp
        195                 200                 205

Cys Lys Asp His Leu Lys Ile Pro Asn Cys His Ile Ile Asp Trp Asn
    210                 215                 220

Asn Lys Glu Glu Ser Tyr Trp Asp Met Cys Leu Met Thr Tyr Cys Lys
225                 230                 235                 240

His Asn Ile Ile Ala Asn Ser Ser Phe Ser Trp Trp Gly Ala Trp Leu
                245                 250                 255

Asn Thr Asn Pro Glu Arg Ile Val Ile Ala Pro Gly Lys Trp Ile Asn
            260                 265                 270

Asp Asp Arg Val Gln Val Ser Asp Ile Ile Pro Ser Asp Trp Ile Cys
        275                 280                 285

Val
```

<210> 12
<211> 287
<212> PRT
<213> Bacteroides fragilis

<400> 12

```
Met Leu Tyr Val Ile Leu Arg Gly Arg Leu Gly Asn Asn Leu Phe Gln
1               5                   10                  15

Ile Ala Thr Ala Ala Ser Leu Thr Gln Asn Phe Ile Phe Cys Thr Val
            20                  25                  30

Asn Lys Asp Gln Glu Arg Gln Val Leu Leu Tyr Lys Asp Ser Phe Phe
        35                  40                  45

Lys Asn Ile Lys Val Met Lys Gly Val Pro Asp Gly Ile Pro Tyr Tyr
    50                  55                  60
```

Lys Glu Pro Phe His Glu Phe Ser Arg Ile Pro Tyr Glu Glu Gly Lys
65 70 75 80

Asp Leu Ile Ile Asp Gly Tyr Phe Gln Ser Glu Lys Tyr Phe Lys Arg
85 90 95

Ser Val Val Leu Asp Leu Tyr Arg Ile Thr Asp Glu Leu Arg Lys Lys
100 105 110

Ile Trp Asn Ile Cys Gly Asn Ile Leu Glu Lys Gly Glu Thr Val Ser
115 120 125

Ile His Val Arg Arg Gly Asp Tyr Leu Lys Leu Pro His Ala Leu Pro
130 135 140

Phe Cys Gly Lys Ser Tyr Tyr Lys Asn Ala Ile Gln Tyr Ile Gly Glu
145 150 155 160

Asp Lys Ile Phe Ile Ile Cys Ser Asp Asp Ile Asp Trp Cys Lys Lys
165 170 175

Asn Phe Ile Gly Lys Arg Tyr Tyr Phe Ile Glu Asn Thr Thr Pro Leu
180 185 190

Leu Asp Leu Tyr Ile Gln Ser Leu Cys Thr His Asn Ile Ile Ser Asn
195 200 205

Ser Ser Phe Ser Trp Trp Gly Ala Trp Leu Asn Glu Asn Ser Asn Lys
210 215 220

Ile Val Ile Ala Pro Gln Met Trp Phe Gly Ile Ser Val Lys Leu Gly
225 230 235 240

Val Ser Asp Leu Leu Pro Val Ser Trp Val Arg Leu Pro Asn Asn Tyr
245 250 255

Thr Leu Gly Arg Tyr Cys Phe Ala Leu Tyr Lys Val Val Glu Asp Tyr
260 265 270

Leu Leu Asn Ile Leu Arg Leu Ile Trp Lys Arg Lys Lys Asn Met
275 280 285

<210> 13
<211> 3075
<212> DNA
<213> Artificial Sequence

<220>

<223> lacY chromosomal construct

<400> 13

atgaccatga ttacggattc actggccgtc gttttacaac gtcgtgactg ggaaaaccct 60

ggcgttaccc aacttaatcg ccttgcagca catcccccct tcgccagctg gcgtaatagc 120

gaagaggccc gcaccgatcg cccttcccaa cagttgcgca gcctgaatgg cgaatggcgc 180

tttgcctggt ttccggcacc agaagcggtg ccggaaagct ggctggagtg cgatcttcct 240

gaggccgata ctgtcgtcgt cccctcaaac tggcagatgc acggttacga tgcgcccatc 300

tacaccaacg tgacctatcc cattacggtc aatccgccgt ttgttcccac ggagaatccg 360

acgggttgtt actcgctcac atttaatgtt gatgaaagct ggctacagga aggccagacg 420

cgaattattt ttgatggcgt taactcggcg tttcatctgt ggtgcaacgg gcgctgggtc 480

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caggatatgt ggcggatgag cggcattttc cgtgacgtct cgttgctgca taaaccgact 660

acacaaatca gcgatttcca tgttgccact cgctttaatg atgatttcag ccgcgctgta 720

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acgctgtgcg accgctacgg cctgtatgtg gtggatgaag ccaatattga aacccacggc 1260

atggtgccaa tgaatcgtct gaccgatgat ccgcgctggc taccggcgat gagcgaacgc 1320

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tgcccgatgt acgcgcgcgt ggatgaagac cagcccttcc cggctgtgcc gaaatggtcc 1560

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agcgaatacc tgttccgtca tagcgataac gagctcctgc actggatggt ggcgctggat 1980

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attgaactgc ctgaactacc gcagccggag agcgccgggc aactctggct cacagtacgc 2100

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Claims

1. Use of a nucleic acid construct, or a bacterial production strain comprising said nucleic acid construct, comprising an isolated nucleic acid encoding a lactose-utilizing α(1,2) fucosyltransferase enzyme, said nucleic acid being operably linked to one or more heterologous control sequences that direct the production of the enzyme in said bacterial production strain, wherein the amino acid sequence of said enzyme encoded by said nucleic acid comprises at least 10% and less than 40% identity to SEQ ID NO: 2.

2. The use of claim 1, wherein said nucleic acid encodes a WblA, FutD, FutN, WbgN, or Bft3/WcfB protein.

3. The use of claim 1 or 2, wherein said heterologous control sequence comprises a bacterial promoter and operator, a bacterial ribosome binding site, a bacterial transcriptional terminator, or a plasmid selectable marker.

4. The use of any one of claims 1 to 3, wherein said production strain comprises *Eschericia coli* K12.

5. The use of any one of claims 1 to 3, wherein said production strain is a member of the *Bacillus*, *Pantoea*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Proprionibacterium*, *Enterococcus*, *Bifidobacterium*, *Sporolactobacillus*, *Micromomospora*, *Micrococcus*, *Rhodococcus*, *or Pseudomonas* genus.

6. The use of any one of claims 1 to 3, wherein said production strain is selected from the group consisting of *Bacillus licheniformis, Bacillus subtilis*, *Bacillus coagulans*, *Bacillus thermophilus*, *Bacillus laterosporus*, *Bacillus megaterium*, *Bacillus mycoides*, *Bacillus pumilus*, *Bacillus lentus*, *Bacillus cereus*, *and Bacillus circulans, Erwinia herbicola* (*Pantoea agglomerans*), *Citrobacter freundii, Pantoea citrea, Pectobacterium carotovorum, Xanthomonas campestris*

Lactobacillus acidophilus, Lactobacillus salivarius, Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus delbrueckii, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus casei, Lactobacillus reuteri, Lactobacillus jensenii, Lactococcus lactis, Streptococcus thermophiles, Proprionibacterium freudenreichii, Enterococcus faecium, Enterococcus thermophiles), *Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium bifidum, Pseudomonas fluorescens* and *Pseudomonas aeruginosa.*

7. The use of any one of claims 1 to 6, wherein the amino acid sequence of said enzyme encoded by said nucleic acid comprises at least 25% and less than 40% identity to SEQ ID NO:2.

8. The use of any one of claims 1 to 7, wherein said nucleic acid encodes a protein selected from the group consisting of *Vibrio cholera* O22 WblA, *Helicobacter bilis* ATCC 437879 FutD, *Bacteroides vulgatus* ATCC 8482 glycosyl transferase family protein (FutN), *Escherichia coli* O55:H7 (str. CB9615) fucosyltransferase (WbgN), and *Bacteroides fragilis* (NCTC) 9343 fucosyl transferase (Bfl3/WcfB).

9. The use of any one of claims 1 to 8, wherein said nucleic acid encodes a protein comprising an amino acid sequence as set forth in SEQ ID NO: 3, 5, 8, 10, or 12.

10. The use of any one of claims 1 to 9, wherein said bacterial production strain comprises a reduced level of β-galactosidase activity, a defective colonic acid synthesis pathway, a mutation in an adenosine-5'-triphosphate (ATP)-dependent intracellular protease, a mutation in the *lacA* gene, and a mutation in the *thyA* gene.

11. The use of any one of claims 1 to 10, wherein an endogenous *lacZ* gene and an endogenous *lacI* gene of said bacterial production strain are deleted or functionally inactivated.

12. The use of any one of claims 1 to 11, wherein said bacterial production strain comprises a *lacIq* gene promoter immediately upstream of a *lacY* gene.

13. The use of any one of claims 1 to 12, wherein an endogenous *wcaJ* gene of said bacterial production strain is deleted or functionally inactivated.

14. The use of any one of claims 10 to 13, wherein said mutation in said ATP-dependent intracellular protease is a mutation in a *lon* gene.

15. The use of any one of claims 1 to 14, wherein said bacterial production strain accumulates intracellular lactose in the presence of exogenous lactose.

16. The use of any one of claims 1 to 15, wherein said bacterial production strain accumulates intracellular guanosine diphosphate (GDP)-fucose.

17. The use of any one of claims 1 to 16, wherein said bacterial production strain comprises the genotype $\Delta ampC::P_{trp}{}^{B}cI$, $\Delta(lacI\text{-}lacZ)::FRT$, $P_{laclq}lacY^{+}$, $\Delta wcaJ::FRT$, $thyA::Tn10$, $\Delta lon:(npt3, lacZ^{+})$.

18. The use of any one of claims 1 to 17 to produce a fucosylated oligosaccharide in said bacterial production strain.

19. The use of claim 18, comprising:

culturing said bacterial production strain in the presence of lactose; and
retrieving a fucosylated oligosaccharide from said bacterium or from a culture supernatant of said bacterium.

20. The use of claim 19, further comprising culturing said bacterium in the presence of tryptophan and in the absence of thymidine.

21. The use of any one of claims 18 to 20, wherein said fucosylated oligosaccharide comprises 2'-fucosyllactose (2'-FL), lactodifucotetraose (LDFT), Lacto-N-fucopentaose I (LNF I), or lacto-N-difucohexaose I (LDFH I).

22. The use of any one of claims 18 to 21, wherein said fucosylated oligosaccharide comprises 2'-fucosyllactose (2'-FL).

FIG. 1

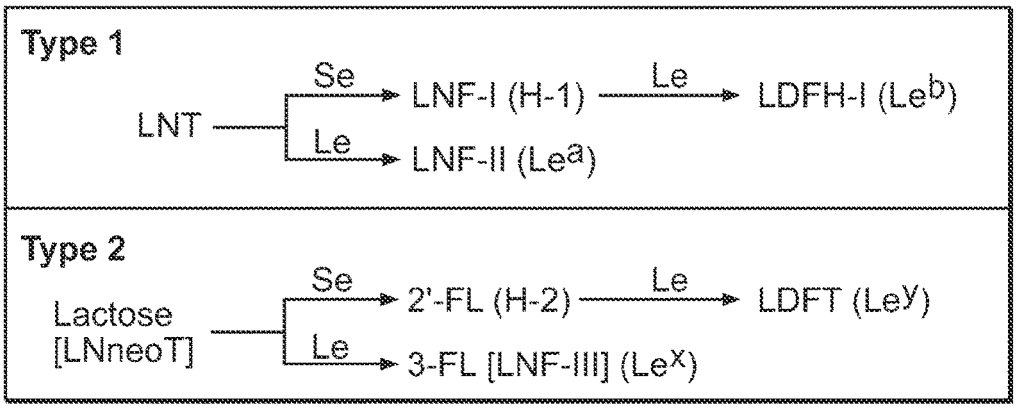

Type 1
LNT
Se
Le
LNF-I (H-1)
LNF-II (Le^a)
Le
LDFH-I (Le^b)
Type 2
Lactose
[LNneoT]
Se
Le
2'-FL (H-2)
3-FL [LNF-III] (Le^x)
Le
LDFT (Le^y)

FIG. 2

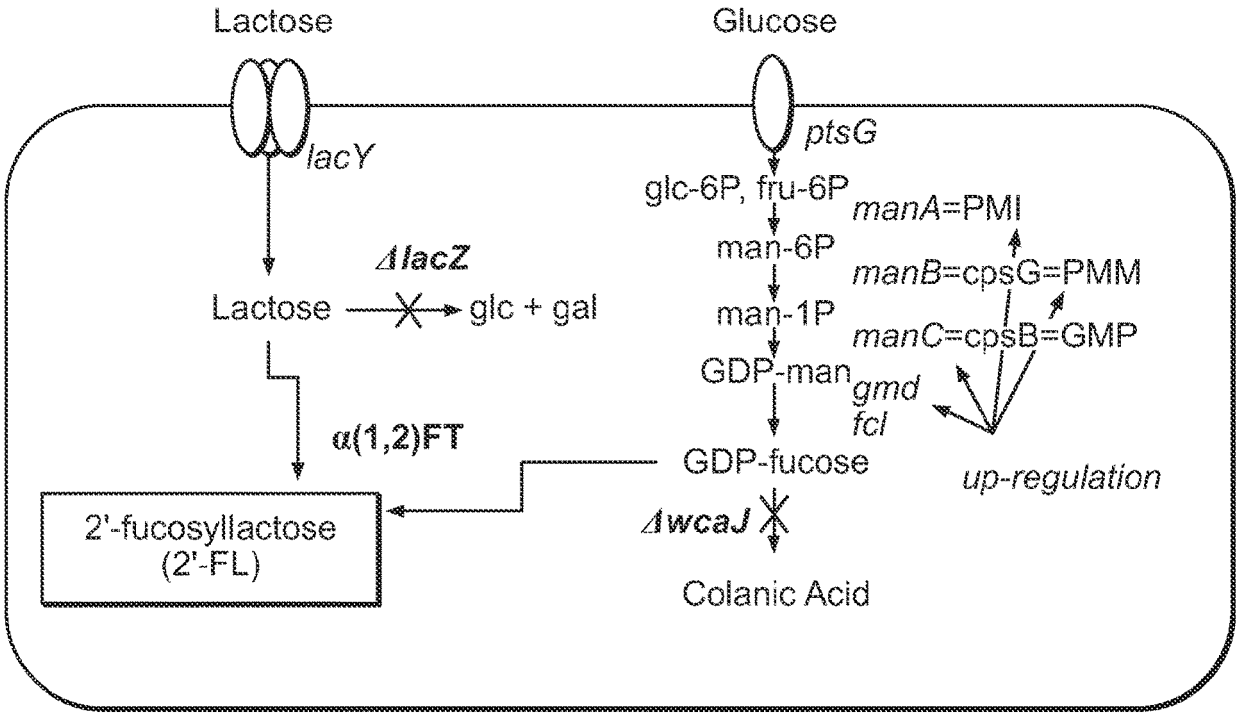

Lactose
Glucose
lacY
ptsG
glc-6P, fru-6P
manA=PMI
man-6P
manB=cpsG=PMM
man-1P
manC=cpsB=GMP
GDP-man
gmd
fcl
ΔlacZ
Lactose
glc + gal
α(1,2)FT
GDP-fucose
up-regulation
2'-fucosyllactose
(2'-FL)
ΔwcaJ
Colanic Acid

FIG. 3A

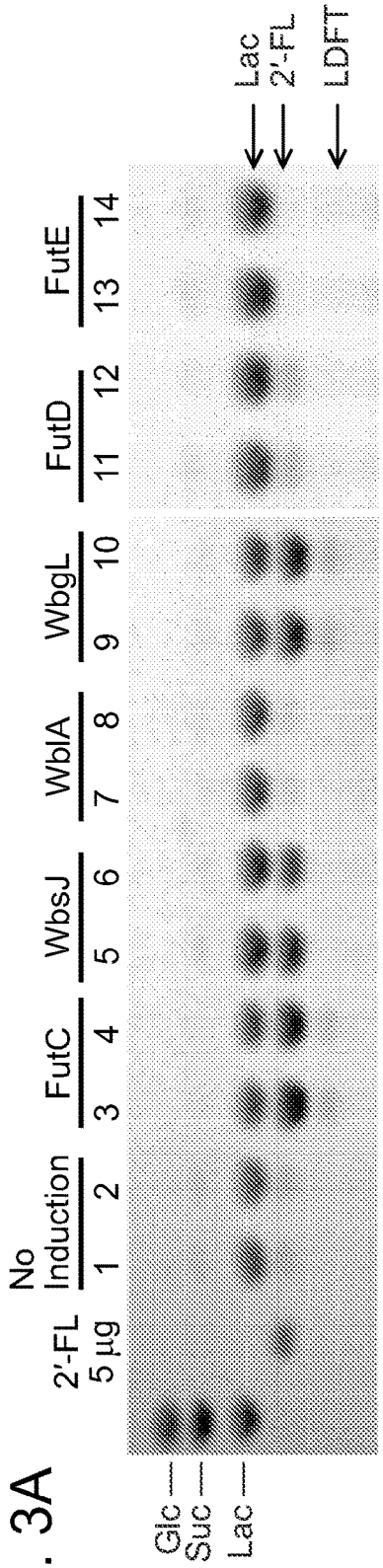


FIG. 3B

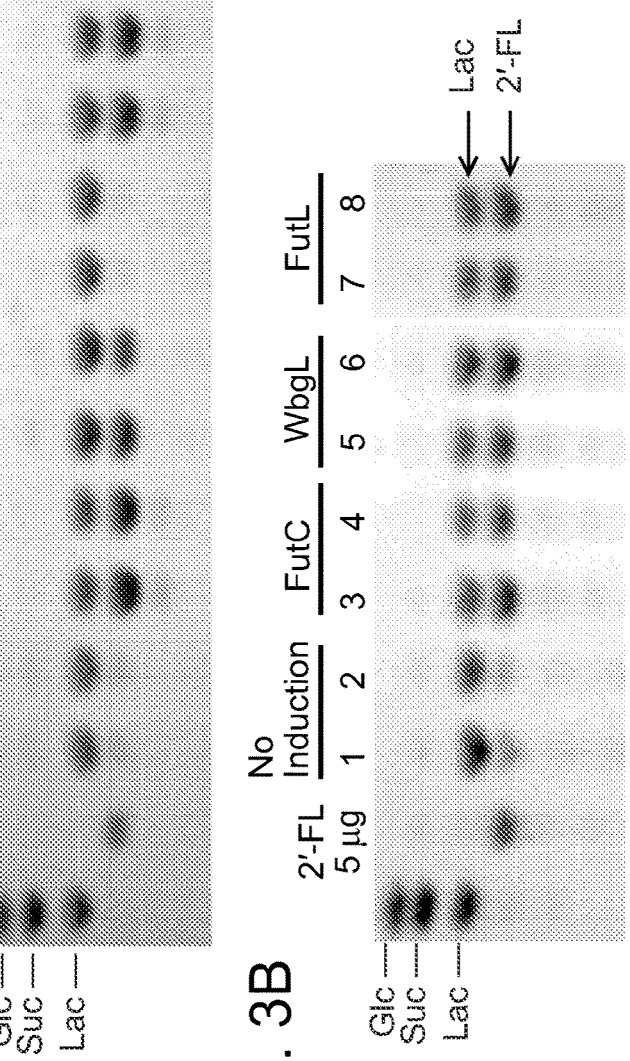


FIG. 3C

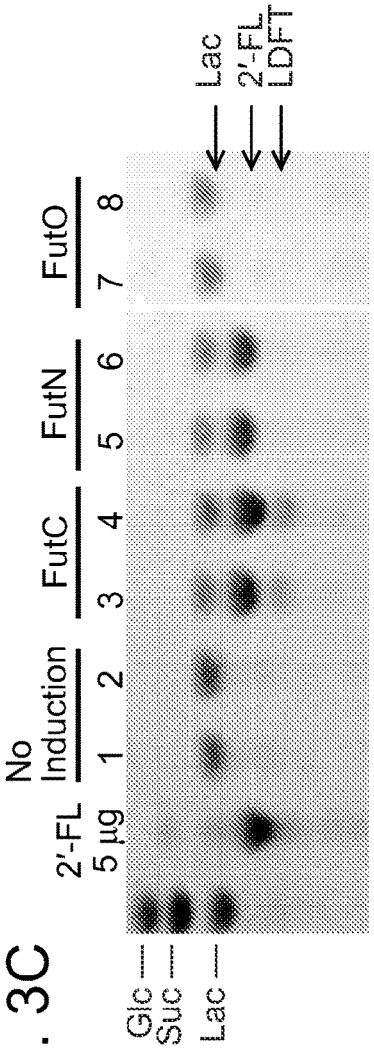


FIG. 4

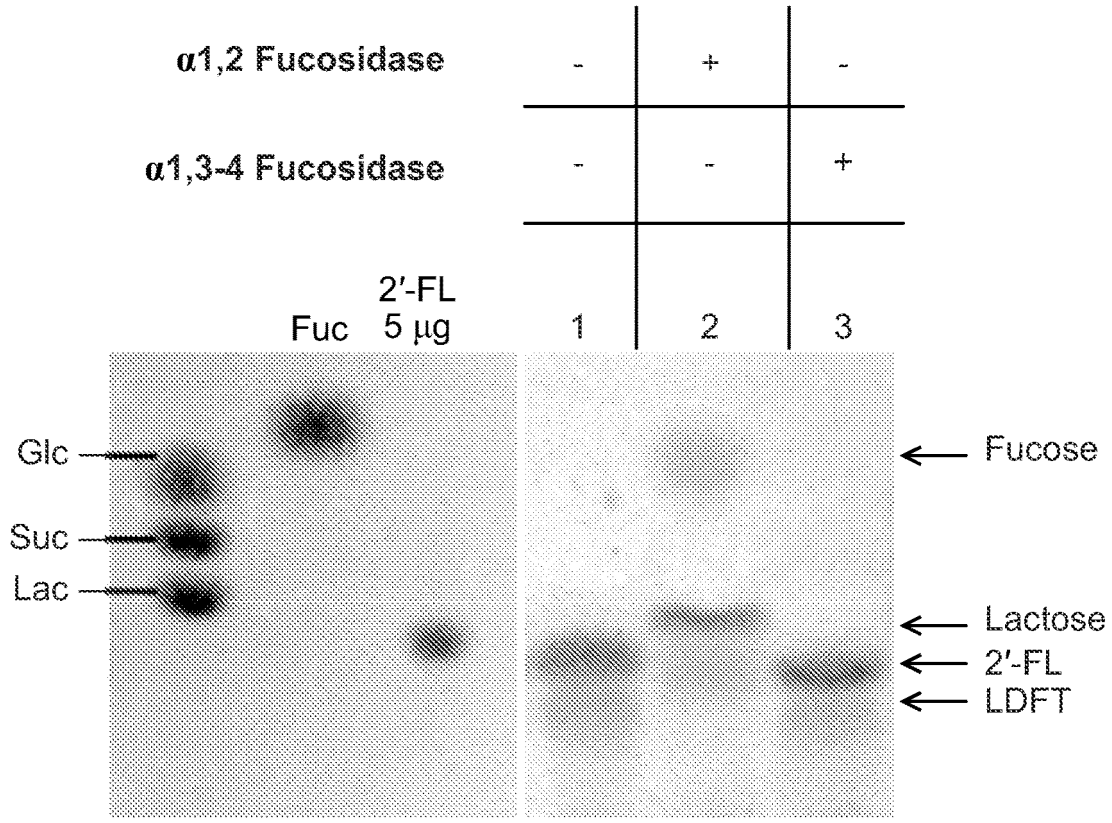

α1,2 Fucosidase
-
+
-
α1,3-4 Fucosidase
-
-
+
Fuc
2'-FL 5 µg
1
2
3
Glc
Suc
Lac
Fucose
Lactose
2'-FL
LDFT

FIG. 5

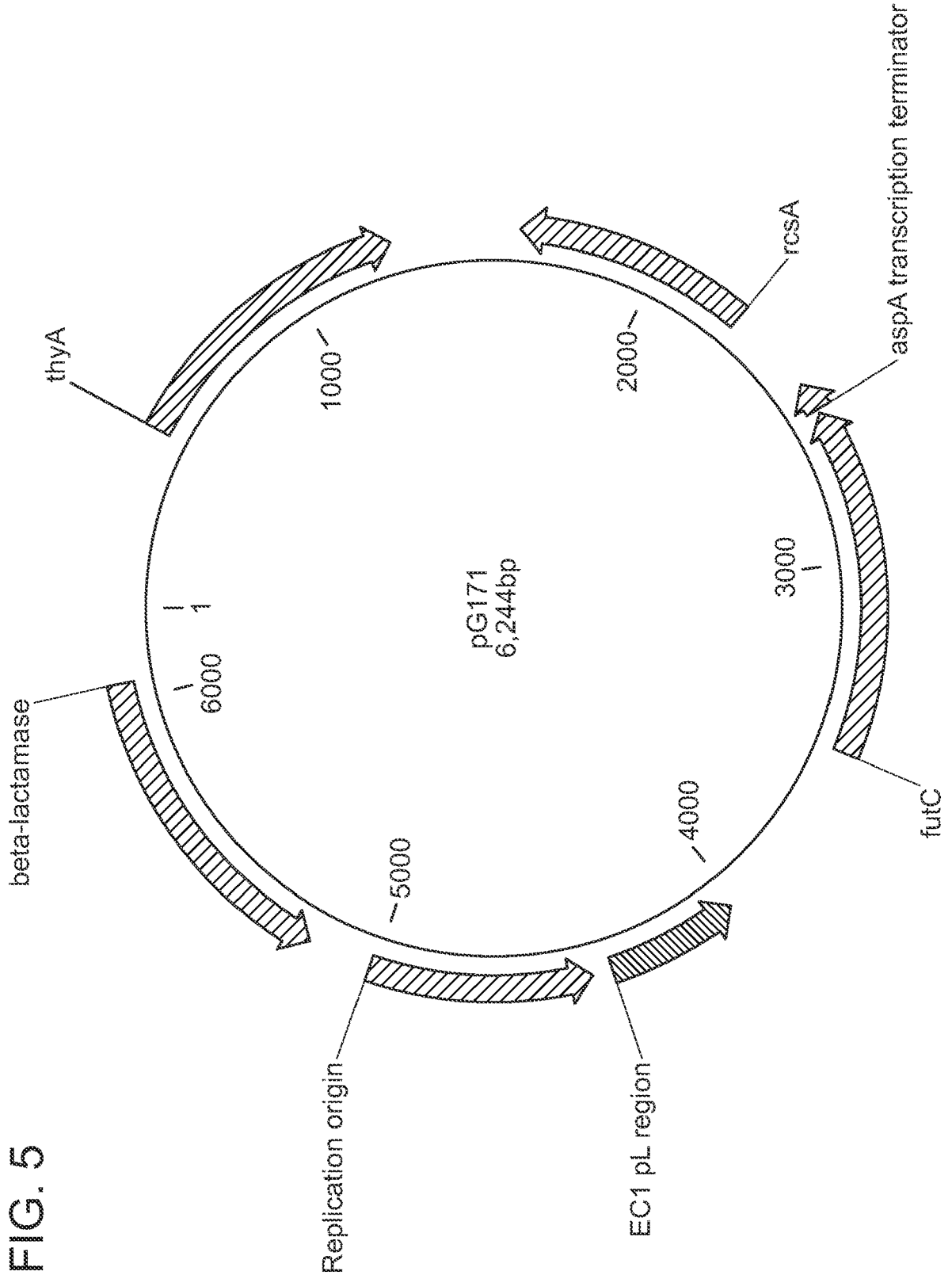

pG171
6,244bp
1
1000
2000
3000
4000
5000
6000
thyA
rcsA
aspA transcription terminator
futC
EC1 pL region
Replication origin
beta-lactamase

FIG. 6

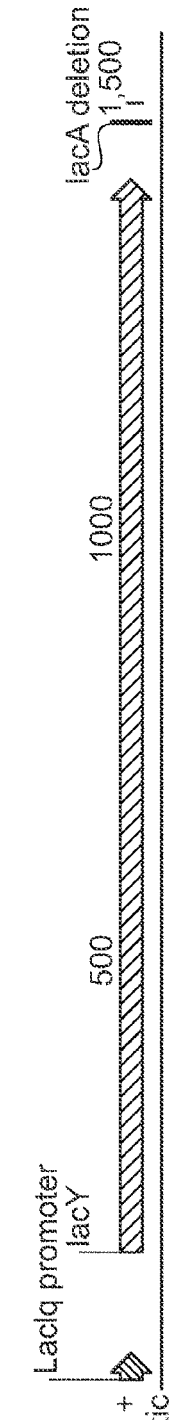


FIG. 7

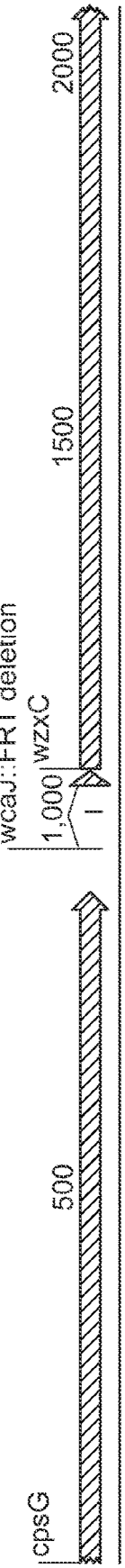


FIG. 8

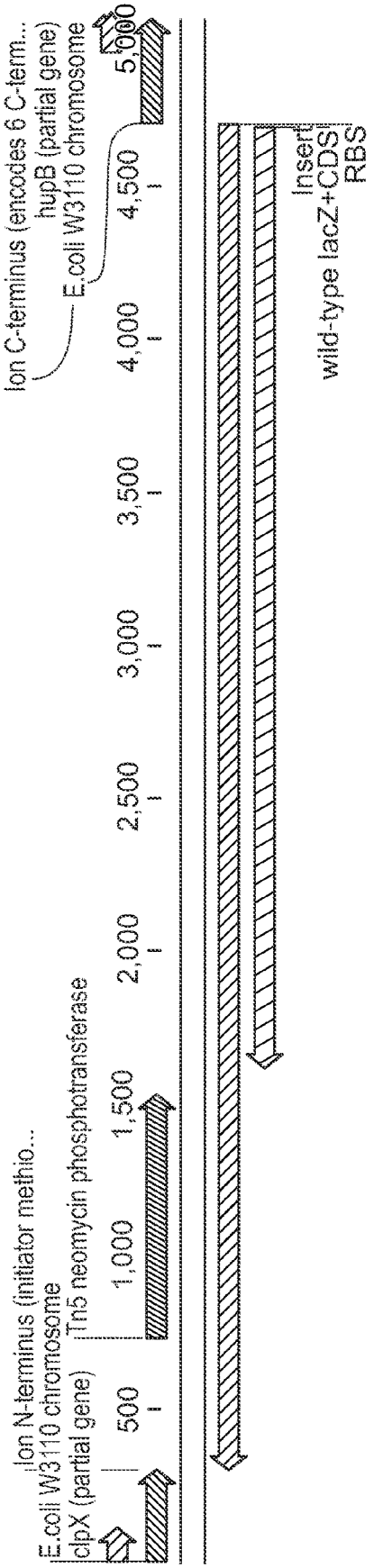


FIG. 9

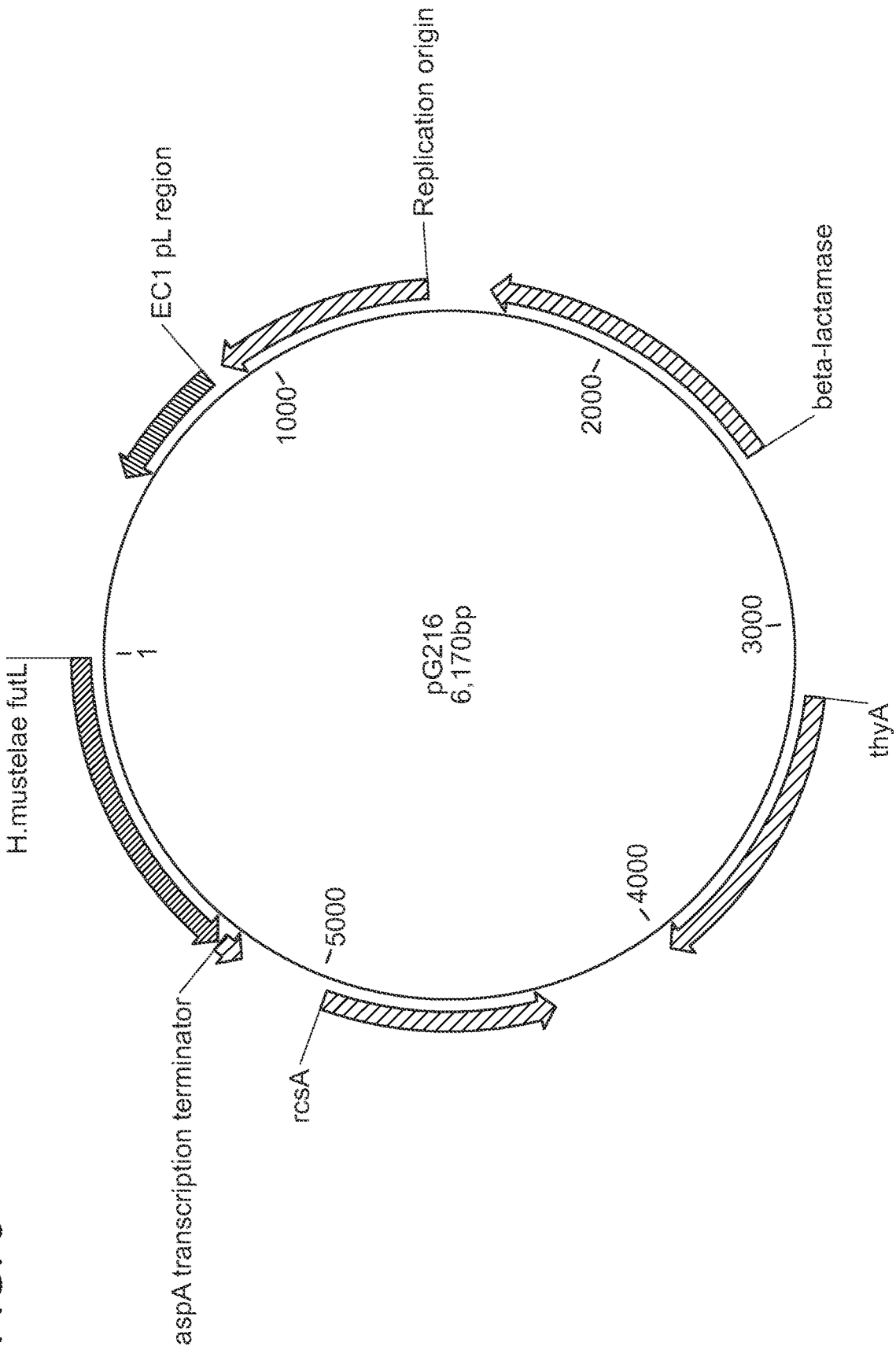

Replication origin
EC1 pL region
beta-lactamase
thyA
rcsA
aspA transcription terminator
H.mustelae futL
1000
2000
3000
4000
5000
1
1
pG216
6,170bp

FIG. 10

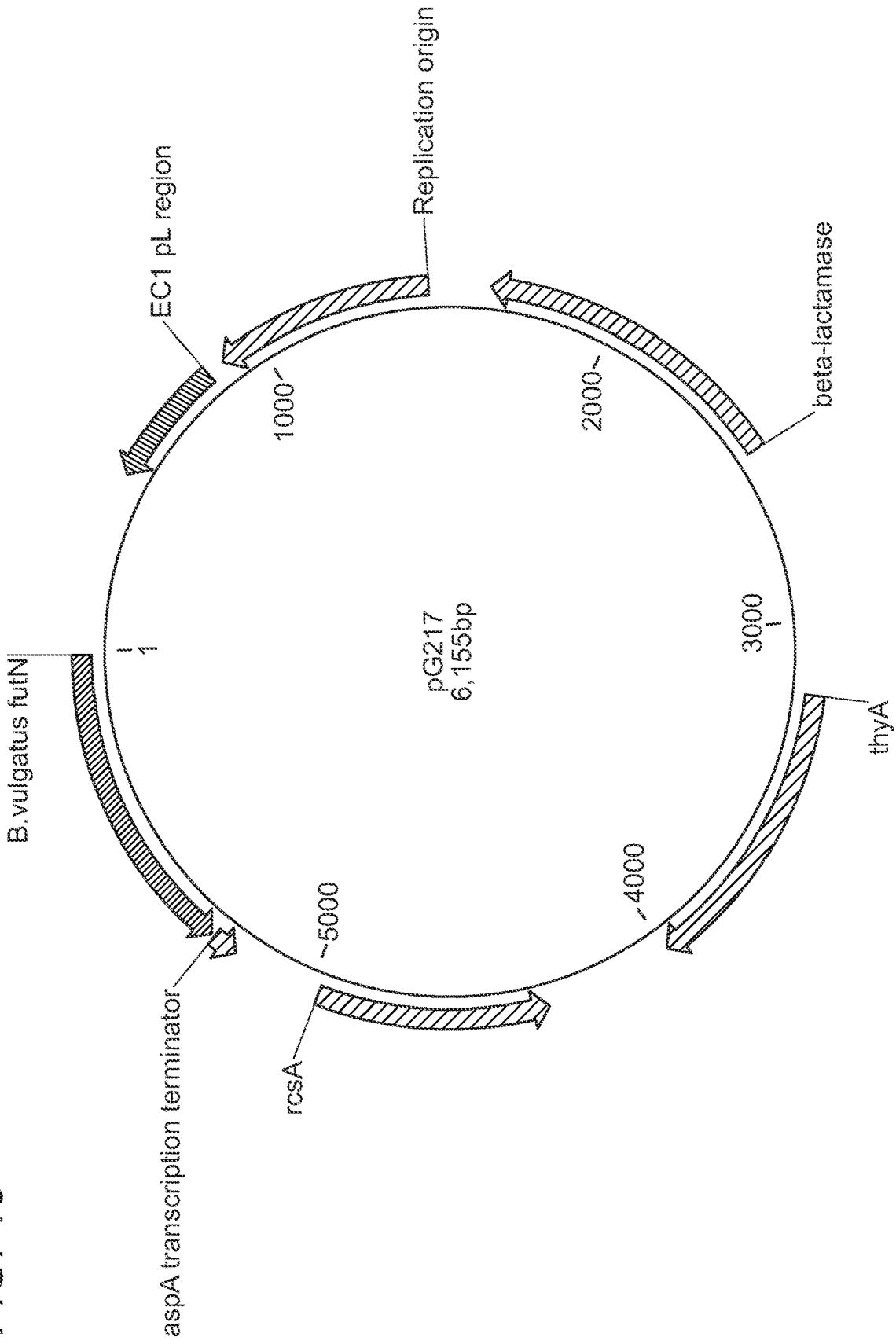
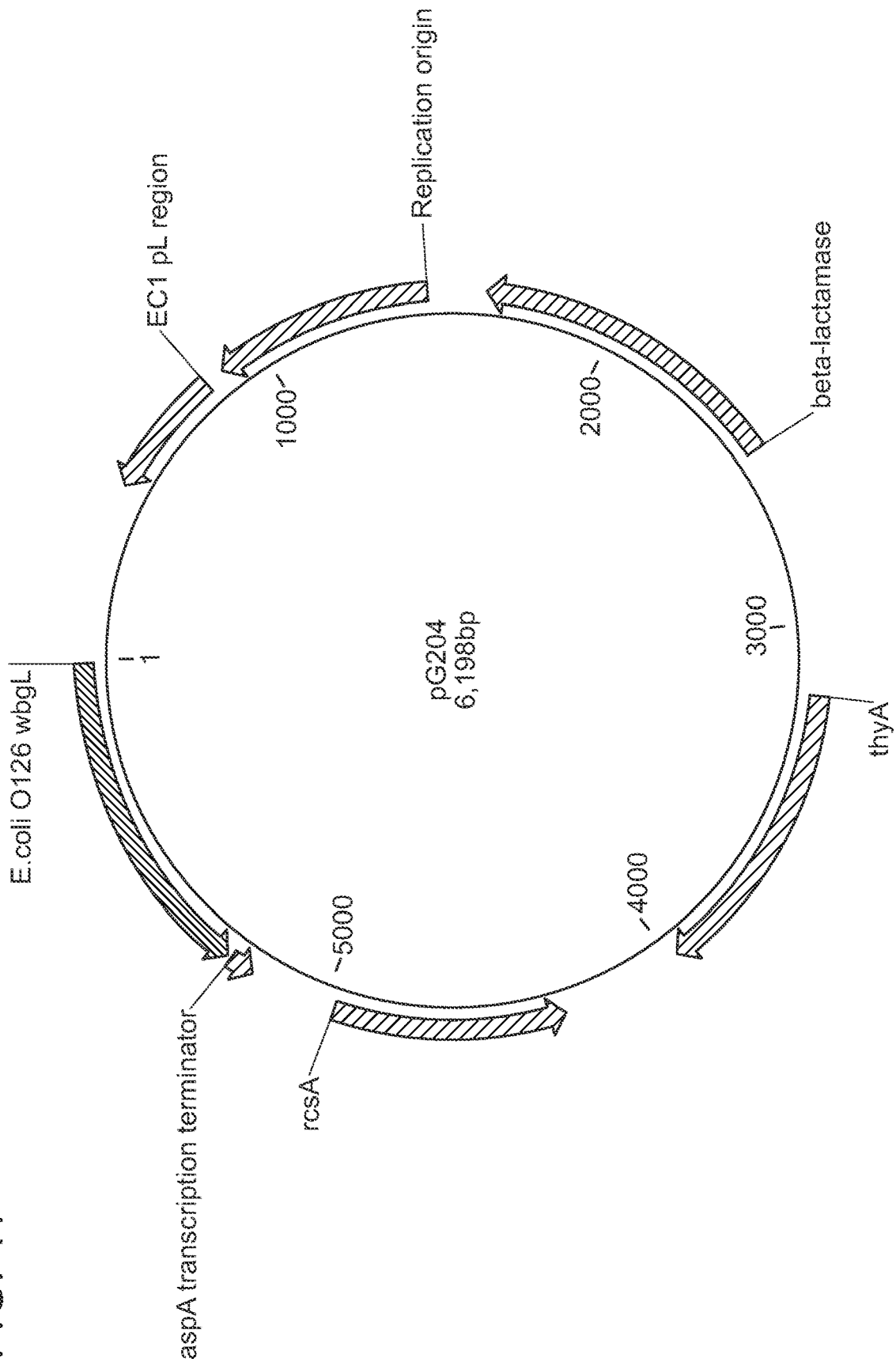

Replication origin
EC1 pL region
beta-lactamase
thyA
rcsA
aspA transcription terminator
B.vulgatus fjfN
pG217
6,155bp
1
1000
2000
3000
4000
5000

FIG. 11


Replication origin
EC1 pL region
beta-lactamase
1000
2000
3000
4000
5000
1
1
pG204
6,198bp
E.coli O126 wbgL
aspA transcription terminator
rcsA
thyA

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EUROPEAN SEARCH REPORT

Application Number
EP 19 17 4435

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	16 October 2019	Vollbach, Silke

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EUROPEAN SEARCH REPORT

Application Number
EP 19 17 4435

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	16 October 2019	Vollbach, Silke

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